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195. UREA AS A PROTEIN SUBSTITUTE IN THE DIET OF YOUNG CATTLE

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(With 1 Figure)

THE nitrogen requirements of the higher animals are obtained chiefly from dietary protein. Certain non-protein nitrogenous substances found in plants can be utilized, but the dividing line at which nitrogenous compounds cease to be a useful source of metabolic nitrogen is not clearly marked and doubtless varies in different species. The final breakdown or excretory products of protein in ruminants as in other mammals consist largely of urea and various ammonium salts, compounds usually considered valueless to animals as a source of nitrogen. Thus in appraising the food value of silage it is usual to regard that nitrogen which has been broken down as far as ammonia during the fermentation process as lost nitrogen. During recent years, however, a number of continental investigators claim to have shown that the nitrogen of urea and certain ammonium salts can be utilized, at least in part, by ruminants. Since the digestive system of these animals is characterized by a temporary storage and fermentation of food in the rumen, it is possible that as a result of micro-biological activity in this part of the alimentary tract a number of otherwise useless substances may be made available. It is well known that certain types of micro-organisms utilize simple nitrogenous compounds in preference to the more complex proteins for building up their tissues, so that the presence of such simple compounds in the rumen may have a protein-sparing action. In addition to this the micro-organisms may build up their own protein from the simple compounds in the rumen. This protein may well be digested farther down the generous length of the intestine, and the hydrolytic products utilized by the ruminant. Assuming for the moment that the claims just mentioned are correct, it is of course true that in this country at present, while ample supplies of relatively cheap high-protein cattle foods are available to supplement the protein-deficient home-grown foods, the economic value of artificially synthesized nitrogenous substances which might be substituted for dietary protein is likely to be small. Nevertheless, synthetic nitrogen, if indeed utilizable as animal food, might in certain circumstances be a useful alternative to imported protein. In some parts of the world where protein foods are scarce and transport conditions difficult, the introduction of nitrogen as the chemically stable compound urea might even now be economically advantageous.

Much experimental work on this subject has been done in Germany, and Ehrenberg and his co-workers have published a number of reports (1, 2, 3, 4, 5, 6) describing the value of ammonium bicarbonate as a protein substitute in the rations of dairy cows. Other experimenters have used urea (7, 8, 9) in a similar manner. In most of these experiments a normal ration has been compared with a ration supplying the same starch or energy values but with part of the protein nitrogen replaced by a non-protein nitrogenous compound. A slight reduction in milk yield or live weight usually accompanied the substitution, and unsatisfactory results were reported when all the protein of the concentrated food of cows was replaced by ammonium bicarbonate (4). Improved nitrogen balances (7) resulted from the addition of urea, and the evidence in many of these experiments suggested the utilization of the nitrogen from non-protein compounds. One great difficulty in the interpretation of the results is the uncertainty of the minimum protein requirements of animals. Mitchell (11) deals with this matter in some detail and concludes that many of the widely used protein standards for cattle allow two or three times as much protein as the minimum requirements. It seemed possible therefore that in many of the German experiments no dietary protein deficiency occurred even when protein replacement was highest. In order to secure evidence of a more convincing nature a threefold experiment was planned in which one group of animals was given a ration which was low in protein but supplied ample energy value, another group was fed a ration supplying the same energy value but more protein, while a third group was fed the low protein ration plus a supplement of urea.

A report of an American investigation was published shortly after the commencement of the present work (10). It describes an experiment in which the arrangement of the diets and the control was somewhat similar to that reported here, except that the number of animals used was much smaller, i.e. only one animal was allocated to each diet. The live-weight increases over 14 weeks were: on the low-protein ration 65 lb., with ammonium bicarbonate 105 lb., with urea supplement 110 lb., and with casein (milk protein) 126 lb.

Plan of experiment

Twenty-one dairy heifers aged 7–17 months were used for the experiment. The nine older animals were divided into three blocks or pens of three animals each. The three animals in each block were matched as well as possible for age, live-weight, breed, etc., they were housed in one pen and diets were allocated by lot as follows: (1) normal-protein ration, (2) low-protein ration, (3) the low-protein ration supplemented with urea in sufficient quantity to supply the same total nitrogen as the normal-protein ration.

The twelve younger animals were divided in a similar manner into three blocks or pens of four animals each. Randomized diet treatments were then allocated as with the older animals and one additional treatment was intro-

duced, viz. the low-protein ration in which a small quantity of starchy food was replaced by pure ground-nut protein, making the total nitrogen content equal to the normal-protein ration.

Thus there were six replications of the normal, low-protein and urea treatments and three replications of the ground-nut-protein treatment.

The animals were housed in pens of a suitable size in a covered yard; they were tied up by day to ensure correct rationing and were loose by night, when they had access to water and the older animals were allowed oat straw. The chemical analysis of the foods was carried out by Dr W. L. Davies and the results are given in Table I.

Table I. *Chemical composition of foods*

	3 samples meadow hay	2 samples oat straw	Mangels	Decorticated ground-nut cake	Flaked maize	Tapioca flour	Ground-nut protein	Urea
	%	%	%	%	%	%	%	%
	As shown by analyses							
Moisture	10·3	12·6	87·2	12·9	12·1	13·2	—	—
Crude protein (N × 6·25)	6·5	2·7	10·9	46·5	9·4	1·3	95·1	287·8
True protein	5·8	2·2	0·5	46·1	9·2	1·3	—	—
Crude fibre	31·9	35·4	0·7	8·8	2·1	3·2	—	—
Soluble carbohydrates (nitrogen-free extract)	45·1	39·9	8·8	21·0	71·7	79·6	—	—
Fat (ether extract)	1·3	1·4	0·04	6·3	3·4	0·4	—	—
Ash	4·9	8·0	2·4	4·5	1·3	2·3	—	—
CaO	0·56	0·70	0·17	0·30	0·05	0·18	—	—
P_2O_5	0·53	0·74	0·05	1·07	0·07	0·20	—	—
	Calculated digestible nutrients							
Crude protein	3·1	0·8	0·5	41·9	7·0	1·0	85·6	—
Crude fibre	16·0	21·2	0·6	0·8	1·2	1·9	—	—
Soluble carbohydrates	27·9	20·3	8·2	17·6	67·4	74·8	—	—
Fat	0·7	0·5	—	5·7	3·2	0·3	—	—
Total digestible nutrients (digestible crude protein plus digestible carbohydrates, plus 2·25 × digestible fat)	48·5	43·5	9·3	*73·1	82·8	78·4	—	—

* According to Wood & Woodman (12) the "gross digestible energy as starch" of decorticated ground-nut cake and flaked maize are 87·4 and 87·3 per 100 lb. respectively.

The rations, the nutrients supplied and the requirements of the animals according to three different authorities (11, 12, 13) are detailed in Table II. The essential differences between the foods of the four rations were (1) the normal-protein ration included 1 lb. per animal per day of decorticated ground-nut cake; (2) in the low-protein ration this was replaced by 1 lb. of flaked maize; (3) the urea ration was the same except for the addition of 0·127 lb. of urea; and (4) in the ground-nut-protein ration the pound of flaked maize was replaced by 0·6 lb. of flaked maize plus 0·4 lb. ground-nut protein.

The initial difference in size of the experimental animals made it impossible to feed them all alike, but all the animals in one pen were assumed to have the

same food requirements based on the average live weight of the pen. The slight differences in the rations of different pens are shown in Table II. At the commencement of the experiment the amount of digestible nutrients fed to all pens slightly exceeded the standard requirements recommended by Morrison (13), but after a period of 1 month slight increases of food became necessary for the twelve younger animals. Thereafter slight increases in the rations of all animals were made at approximately monthly intervals, till by

Table II. *Rations and nutrients supplied per animal per day (lb.)*

Identifying number of pen	19	20	21	22	23	24
Number of animals in pen	3	3	3	4	4	4
Mean initial live weight of animals (lb.)	535	604	493	441	367	315
Foods in ration: Meadow hay	6	6	6	6	6	6
Oat straw	6	7	5	4	2	—
Mangels	20	20	20	10	10	10
Tapioca flour	0·75	1	0·75	1	1	1
Minerals: Steamed bone flour	0·0178	0·0178	0·0178	0·0178	0·0178	0·0178
Ground chalk	0·0178	0·0178	0·0178	0·0178	0·0178	0·0178
Common salt (NaCl)	0·0178	0·0178	0·0178	0·0178	0·0178	0·0178
Nitrogenous or other supplement added:						
To normal-protein group:						
Decorticated ground-nut cake	1	1	1	1	1	1
To low-protein group:						
Flaked maize	1	1	1	1	1	1
To urea supplement group:						
Flaked maize	1	1	1	1	1	1
Urea	0·127	0·127	0·127	0·127	0·127	0·127
To ground-nut protein group:						
Flaked maize	—	—	—	0·6	0·6	0·6
Decorticated ground-nut protein	—	—	—	0·4	0·4	0·4
Total digestible nutrients:						
Estimated requirements *vide* Morrison (13)	8·0	8·7	7·5	6·9	6·0	5·3
Supplied by ration	8·8	9·4	8·5	7·2	6·3	5·5
Digestible crude protein:						
Estimated requirements *vide* Wood (12)	1·25	1·30	1·20	1·15	1·05	1·00
Estimated requirements *vide* Morrison (13)	1·04	1·12	1·00	0·96	0·88	0·80
Estimated requirements *vide* Mitchell (11)	0·44	0·43	0·44	0·45	0·47	0·48
Supplied by normal-protein ration	0·76	0·77	0·75	0·70	0·68	0·67
Supplied by low-protein ration	0·41	0·42	0·40	0·35	0·33	0·32
Supplied by urea supplement ration	0·41	0·42	0·40	0·35	0·33	0·32
Supplied by decorticated ground-nut-protein ration	—	—	—	0·66	0·64	0·63
Crude protein:						
Supplied by normal-protein ration	1·21	1·24	1·18	1·07	1·02	0·96
Supplied by low-protein ration	0·84	0·87	0·81	0·70	0·65	0·59
Supplied by urea supplement ration	0·84	0·87	0·81	0·70	0·65	0·59
Supplied by ground-nut-protein ration	—	—	—	1·05	1·00	0·94
Weight of nitrogen supplied by urea	0·058	0·058	0·058	0·058	0·058	0·058
Weight of crude protein needed to supply the same weight of nitrogen	0·38	0·38	0·38	0·38	0·38	0·38

the end of the experiment all animals were receiving 2–3 lb. extra straw per day, the nine older animals 7lb. extra mangels and ½ lb. extra flaked maize, while the twelve younger animals were receiving 1 lb. extra flaked maize and 10 lb. extra mangels. The extra protein intake brought about by these changes was small and applied equally to the different dietary treatments.

Table II shows the protein content of each ration expressed as digestible crude protein and as crude protein. The ration described as "normal protein" supplied rather less digestible crude protein than the standards of Wood or Morrison, while the digestible crude protein of the "low-protein" ration was slightly lower than the minimum requirements noted by Mitchell (11). There was a difference of 0·37 lb. crude protein between the two rations, and it was therefore expected to obtain an appreciable difference in their growth-rate response.

The animals were grouped and housed in preparation for the experiment about 3 weeks before the separate feeding was introduced. During this period preliminary live weights and measurements were taken and the rations were the same as noted in Table II, except that 1 lb. of mixed flaked maize and decorticated ground-nut cake was fed instead of 1 lb. of one of these foods. The separate experimental rations and urea feeding was commenced on 25 December 1937; unfortunately the ground-nut protein was not available on this date, and the three animals on this diet were given the "low-protein" ration till 1 February when a change was made to the ration noted in Table II.

Consumption of the rations by all animals was almost complete, the only exceptions occurring during the early stages of the experiment when occasional feeds were only partly eaten. No animals remained "off feed" for more than a day or so, but it was noticeable throughout the experiment that the urea-fed animals ate their concentrates containing the urea more slowly than the other animals.

Straw was used for bedding and, when fresh, small amounts of this were eaten; on the other hand, a little of the straw given in the ration was sometimes refused, and it is reasonably certain that the intake of straw did not exceed the amounts noted in Table II.

The appetite of all animals appeared to be well satisfied. Other workers have reported that animals given large quantities of urea usually develop pronounced diuresis. No measurement of the output of urine was possible in this experiment, but observation failed to detect any difference in excretion between the groups.

Records included live weights, body measurements, observed fatness, skin handling properties and photographs.

Results

The initial and final live weights were determined on 19 December 1937 and 10 May 1938 respectively (mean of three days' weighing), and the live-weight increases were calculated for the intervening 142 days. The results

relating to individual animals are given in Table III together with their ages. Table IV gives a classified statement of the daily live-weight increases of the animals on each dietary treatment, the nine older animals being shown separately from the twelve younger ones; the three dietary treatments with six replications are also shown.

Table III. *Live-weight increases and other details of animals*

Identifying no. of animals	Breed	Group	No. of pen for housing	Initial age on 19. xii. 37	Initial live wt. on 19. xii. 37	Final live wt. on 10. v. 38	Live-wt. increase during winter period	
							In 142 days	Per day
				days	lb.	lb.	lb.	lb.
40	Guernsey	Normal protein	19	514	551·3	701·3	150·0	1·06
43	Dairy Shorthorn	,,	20	467	590·0	781·3	191·3	1·35
50	,,	,,	21	380	518·7	742·7	224·0	1·58
Mean of 3 older normal-protein animals				454	553·3	741·8	188·4	1·33
45	Guernsey	Low protein	19	447	522·0	639·3	117·3	0·83
41	Dairy Shorthorn	,,	20	482	654·7	786·7	132·0	0·93
49	,,	,,	21	382	479·3	644·0	164·7	1·09
Mean of 3 older low-protein animals				437	552·0	690·0	138·0	0·97
42	Guernsey	Urea	19	478	531·3	660·0	128·7	0·91
46	Dairy Shorthorn	,,	20	389	568·0	748·0	180·0	1·27
48	,,	,,	21	385	480·7	654·7	174·0	1·23
Mean of 3 older urea-fed animals				417	526·7	687·6	160·9	1·13
51	Guernsey	Normal protein	22	361	465·3	652·0	186·7	1·31
55	Dairy Shorthorn	,,	23	293	315·3	514·0	198·7	1·40
62	,,	,,	24	211	293·3	523·3	230·0	1·52
Mean of 3 younger normal-protein animals				288	358·0	563·1	205·1	1·44
52	Dairy Shorthorn	Low protein	22	325	418·7	526·7	108·0	0·76
54	,,	,,	23	293	358·0	483·3	125·3	0·88
61	,,	,,	24	221	329·3	522·7	193·4	1·36
Mean of 3 younger low-protein animals				280	368·7	510·9	142·2	1·00
57	Dairy Shorthorn	Urea	22	284	420·7	599·3	178·7	1·26
53	,,	,,	23	319	394·0	622·7	228·3	1·61
59	Guernsey	,,	24	230	320·2	481·3	161·3	1·14
Mean of 3 younger urea-fed animals				278	378·2	567·8	189·4	1·33
47	Dairy Shorthorn	Ground-nut protein	22	388	458·7	678·7	220·0	1·55
56	,,	,,	23	286	402·0	621·3	219·3	1·54
60	,,	,,	24	228	316·0	524·0	208·0	1·46
Mean of 3 ground-nut-protein animals				301	392·2	608·0	215·8	1·52

Table IV. *Mean daily live-weight increases during 142 days winter-feeding period*

	Dietary treatment					
	Normal protein lb.	Low protein lb.	Urea supplement lb.	Ground-nut protein lb.	Mean lb.	Standard error lb.
9 older animals (groups of 3)	1·33	0·97	1·13	—	1·14	0·050
12 younger animals (groups of 3)	1·44	1·00	1·33	1·52	1·32	0·131
18 animals (groups of 6)	1·39	0·99	1·23	—	1·20	0·073

The standard errors shown in the table were obtained from the calculation of variance by Fisher's method (14) which proved significant differences between the dietary treatments of the nine older animals and the eighteen animals but not for the twelve younger animals. A difference between two diets which is at least three times the standard error may be accepted as proof of statistical significance. On this basis there are significant differences between all three diets fed to the nine older animals; the urea supplement with live-weight increases of 1·13 lb. per day is significantly better than the low-protein diet (0·97 lb. per day), and the normal-protein diet (1·33 lb. per day) is significantly better than the urea supplement. The differences between the various groups among the twelve younger animals are not significant. They correspond closely with the older animals except that they show a greater response to the addition of nitrogen in any form. According to Mitchell's standards (11) the low-protein group of the younger animals was more deficient in protein than any other group, but the fact that even this group showed reasonably good growth suggests that the protein intake was not seriously deficient. Minimum requirements are not necessarily the optimum protein standards for maximum growth, and if the animals on the low-protein diet were receiving enough protein to meet essential needs, it can be stated that a higher level of protein intake provided an extra stimulus to growth. The group receiving ground-nut protein showed even better live-weight increases than the normal-protein group, but a larger experiment would be necessary to decide whether this advantage was fortuitous or real.

The results from the eighteen animals showed a significant advantage of the urea supplement (1·23 lb. per day) over the low-protein group (0·99 lb. per day), but the advantage of the normal-protein group (1·39 lb. per day) over the urea supplement was of doubtful significance. The live-weight increases therefore prove with reasonable certainty either that the urea was used as a source of nitrogen or that it exerted a protein-sparing action or possibly that both these alternatives were in action simultaneously.

Only the initial and final weighings have been used in the calculation of results, but the growth of the animals was recorded by regular weighings on three consecutive days each month during the course of the experiment.

The results of each dietary treatment are shown in Fig. 1 and the most outstanding feature is the regularity of growth of all groups. Further weighings of the animals were made on completion of the experiment after 2 days' and after 1 month's grazing. These weighings were taken in order to note whether any variation occurred among the different dietary groups in their response to a ration of grass. In view of the fact that the conclusions to be drawn from the experiments were not affected by these additional live weights, they have been omitted to avoid unnecessary complication of the results.

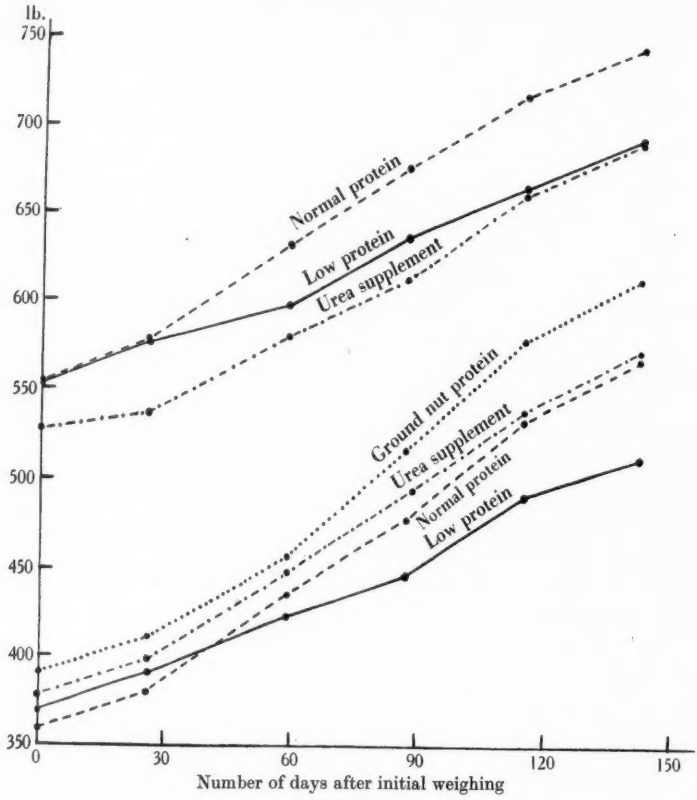

Fig. 1. Live weights of each group.

The body measurements recorded were height at withers, depth of chest and chest girth. The mean initial measurements of each group and the increment during the course of the experiment are shown in Table V. The difficulty of measuring young active animals caused considerable variation in the body measurements, and the increments of height at withers bear little relationship to the live-weight increases; both chest measurements, however, are closely correlated with the live-weight increases of the various groups. The group differences do not approach statistical significance.

Table V. *Body measurements, skin and fatness*

Group	Normal protein		Low protein		Urea supplement		Ground-nut protein	
No of animals in group ...	6		6		6		3	
	Mean initial measurements	Increment during exp. period	Mean initial measurements	Increment during exp. period	Mean initial measurements	Increment during exp. period	Mean initial measurements	Increment during exp. period
Height at withers, in.	41·5	3·05	41·2	3·41	41·8	3·03	39·9	3·84
Depth of chest, in.	20·2	2·00	20·1	1·14	20·4	1·79	18·5	2·40
Chest girth, in.	52·5	6·27	52·5	5·02	52·8	5·49	49·2	7·27
Skin-handling properties judged by touch recorded numerically	—	0·67	—	1·00	—	0·50	—	1·67
Observed fatness recorded by numerical estimate	—	0·50	—	−0·16	—	0·50	—	1·00

The skin-handling properties of each animal were recorded by a numerical estimate based on touch at the beginning and end of the experiment. The results shown in Table V show a slight improvement in all groups during the course of the experiment. The differences between groups are not statistically significant, but the general results reflect the opinion of all observers, viz. that every animal looked remarkably healthy at the end of the experiment. Fatness of each animal as observed and recorded by a numerical estimate was recorded at the beginning and end of the experiment. The results are noted in Table V and the comparisons between dietary treatments agree remarkably well with the live-weight increases. The "low-protein" group was recorded as losing a little flesh during the experiment, but all other groups showed noticeable improvement.

Blood samples were taken from each animal at two stages of the experiment, the first was 4 weeks and the second 14 weeks after the commencement of urea feeding. These samples were analysed for urea and non-protein nitrogen by Dr S. J. Folley. The mean and standard deviations of each group are given in Table VI. The mean values were very similar in the normal-protein and urea groups, but the low-protein group yielded significantly lower values for urea nitrogen though not for total non-protein nitrogen.

Table VI. *Total non-protein and urea nitrogen content of the blood expressed as mg. per* 100 *ml. blood*

	Total non-protein nitrogen		Urea nitrogen		Urea nitrogen	
Date of sampling	5. iv. 38		1st test 25. i. 38		2nd test 5. iv. 38	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Normal-protein group	42·7	7·63	15·6	2·36	17·7	2·99
Low-protein group	41·2	5·69	12·6	1·88	14·4	3·26
Urea-fed group	41·4	4·18	17·2	5·59	17·3	3·39
Ground-nut-protein group	40·6	3·06	16·3	5·92	18·7	3·15

Summary

1. A 142-day feeding experiment with twenty-one dairy heifers aged 7–17 months is described.

2. The addition of 0·127 lb. per animal per day of urea to a diet supplying a limited amount of protein resulted in an extra daily live-weight increase of 0·24 lb. per day. This increase was statistically significant and the animals appear therefore to have utilized the urea nitrogen in their metabolism.

3. Animals receiving the same quantity of additional nitrogen in the form of protein showed slightly better live-weight gains than those receiving the urea; although the difference was not significant it seems probable that the protein was a more efficient nitrogen supplement than urea.

Acknowledgement

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196. THE INFLUENCE OF VARIOUS FODDER SUPPLEMENTS ON THE PRODUCTION AND THE NUTRITIVE VALUE OF WINTER MILK

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(With 8 Figures)

It is universally acknowledged that the nutritive quality of cow's milk is not constant throughout the year but that it varies with the season, summer milk being in this respect superior to milk produced under winter conditions.

The voluminous literature of recent years dealing with the nutritive value of milk and its seasonal changes has been reviewed by one of us (Kon(1, 2, 3)), and references to relevant papers will be found in those reviews. The difference in nutritive value is largely due to seasonal alterations in the diet of the cow, with which, for example, the vitamin A and carotene content of milk is intimately bound. In England the marked difference in the composition of the food between abundant summer grazing and the stall-feeding period is reflected in the much higher vitamin A and carotene values of summer milk. The fluctuation in the vitamin D content of milk is, however, caused not so much by changes in fodder as by seasonal differences in the ultra-violet activity of the sun and in the time spent by cows outdoors.

Certain other vitamins are less affected by food or season. The vitamin C content of herd milk varies but little throughout the year. Vitamin B_1 is also probably fairly constant. The most recent work on lactoflavin suggests that it may be more variable.

Other less well-defined essential factors in milk have also been found to vary with the season, and the most important findings in this respect have been reported by Hart, Elvehjem and their collaborators, of Wisconsin, whose work has been reviewed by Kon(2, 3).

It is now well known that the nutritive property of winter milk can be improved, at least in respect of certain factors, by addition to the normal ration of such fodders as artificially dried grass, A.I.V. and other silages, etc.

The chief object of the experiments described in the present paper was to test the effect on the nutritive value of winter-produced milk of a number of foods which are in common use on farms or which might be regarded as possible alternative foods.

Plan of experiment

Twelve cows divided into four groups of three cows were used for the experiment: each of the four groups was given a different food supplement, and the effect on the live weight and on the yield, composition and nutritive

value of the milk was noted. The four food supplements tested were mangels, kale, artificially dried grass and sprouted maize. The ration containing mangels was regarded as the "control" and was fed to all cows during the "control" periods.

The experiment, begun in December 1936, was divided into four periods:

Period 1. "The preliminary control period", 2 December 1936 to 5 January 1937 (5 weeks), when all the cows received the same control ration, i.e. mangels supplement.

Period 2. "The experimental period", 6 January to 25 March 1937 (11 weeks), when different fodder supplements were fed to each group of cows.

Period 3. "Second control period", 26 March to 2 May 1937 (5 weeks), when all cows received the same ration as during period 1.

Period 4. "Grazing period", 3 May onwards, when the diet of all cows consisted chiefly of fresh green grass.

It was necessary to make one exception to the general programme, viz. the change from period 1 to period 2 for the sprouted maize group of cows was delayed 9 days owing to late delivery of the cabinet used for sprouting the maize.

Dairy Shorthorn cows were used, and details of their age, stage of lactation, fodder supplement group, etc., are given in Table I.

Table I. *Particulars of the experimental cows*

Name of cow	Fodder supplement	No of calvings	Date of last calf	Milk yield during week ending 3 Jan. 1937 lb.	Date of effective service	Initial live weight lb.
Winsome 13	Mangels (control)	1	26. xi. 36	188¾	N.S.	1060
Welcome 7	,,	5	29. xi. 36	326¼	23. iv. 37	1424
Doris 3	,,	2	9. xi. 36	260	20. ii. 37	1088
Lottie 10	Kale	1	1. xii. 36	156½	N.S.	1008
Peach 9	,,	3	26. xi. 36	217¼	N.S.	1476
Clara 4	,,	3	4. xii. 36	258¾	5. v. 37	1292
Puppet	Artificially dried grass	1	24. ix. 36	145½	9. i. 37	944
Campion 2	,,	8	24. xi. 16	276¾	19. iv. 37	1176
Clara 3	,,	3	23. ix. 36	153¼	N.S.	1260
Winsome 12	Sprouted maize	1	15. ix. 36	170½	N.S.	1110
Flora 31	,,	2	2. xii. 36	283¼	19. iii. 37	1128
Lena 4	,,	1	27. x. 36	129½	N.S.	1184

To allot the cows to their groups the twelve animals were first divided into three lots of four cows, each lot of four being as nearly alike as possible in age, stage of lactation and milk yield. Then by lot, one cow from each four was allocated to each diet. This provided reasonably satisfactory groups except for an inequality in the fat percentage of the milk of two groups. In order to provide milk of constant fat content for tests of nutritive value by rat feeding, the grouping of two cows was changed at the end of the preliminary control period. Table I shows the ultimate grouping. This change resulted in a high initial milk yield of the mangel or control group and a low initial milk yield of the sprouted maize group, but otherwise is believed not to have affected the results.

During the first three periods of the experiment all the cows were housed.

The quantity of food supplement used in the rations was considered reasonable under practical conditions, and the exact weights are shown in Table II. The differences in the nutrients and protein supplied by the various supplements were balanced by adding varying quantities of other ingredients, but foods likely to supply vitamin A or its precursors, e.g. hay and maize, were equalized as far as possible.

The nutrients in the ration of each cow supplied about 13 lb. starch equivalent including 2 lb. protein equivalent which was regarded as sufficient for maintenance and the first 2 gal. of milk.

Table II shows the quantity of food per cow per day used for each of the four groups of cows.

Table II. *The rations of the experimental cows*

	Mangel group (control) lb.	Kale group lb.	Artificially dried grass group lb.	Sprouted maize group lb.
Meadow hay (poor quality)	14	14	10	14
Maize (meal or flaked)	1½	1½	1½	1½
Sugar-beet pulp	—	2	—	—
Other concentrated foods	6	4	—	6
Mangels (white fleshed)	42	—	—	—
Thousand headed kale	—	46	—	—
Artificially dried grass	—	—	14	—
Sprouted maize (weighed as dry maize before sprouting)	—	—	—	4
Dry matter	24·0	23·4	23·4	21·0
Starch equivalent	13·0	13·0	13·0	13·2
Protein equivalent	2·1	2·1	2·3	2·2

Cows yielding more than 2 gal. of milk per day were given additional concentrated foods, the amount varying from 3⅓ to 4 lb. per gallon of milk according to the composition of the milk. These concentrates consisted of a mixture of ground-nut cake 16 %, soya-bean cake 15 %, maize meal 22·5 %, wheat offals 12 %, rice bran 22·5 %, molasses 10 %, and minerals 2 %. This mixture was pressed into cubes and contained 65·3 % of starch equivalent and 16·9 % of protein equivalent.

The chemical composition of the four experimental foods was estimated by Dr W. L. Davies, and the results are given in Table III.

Table III. *The chemical composition of additional fodders*

	Mangels (white fleshed)	Kale (thousand headed)	Artificially dried grass	Sprouted maize
Water	89·4	80·3	13·0	62·8
Crude protein	1·0	2·0	19·0	4·1
Oil	0·1	0·2	2·4	2·0
Carbohydrates: Soluble	8·1	12·2	37·8	29·2
Fibre	0·7	3·9	19·8	1·3
Ash	0·7	1·4	8·0	0·6
	100·0	100·0	100·0	100·0

The mangels were a white-fleshed intermediate variety from a crop of normal weight—30 tons per acre. The thousand-headed kale was from a normal yielding crop. The artificially dried grass was a high-grade sample from a good permanent pasture cut on 16 May 1936, when 6–8 in. high. It was wilted in small heaps in the field for 24 hr. and dried in a tray type of dryer at 160° C. for 30 min. The colour of the grass was dark green. It was packed in high-pressure bales of 80 lb. with paper covers and stored in a closed barn.

The "sprouted" maize consisted of a high grade feeding sample of yellow maize sprouted in a specially constructed cabinet heated to about 75° F. A patent nutrient solution was supplied and the maize sprouted for 10 days, light being admitted from about the seventh to the tenth day. By this time the leafy shoots on the maize were about 9 in. long and the whole product, grain, shoots and roots, was fed to the cows. The germination capacity of the maize was only about 66 %. This was abnormally low but appeared to apply to all samples of feeding maize which were on the market at the time. Under the conditions of the experiment only about 33 % of the seeds actually germinated in the sprouting trays. The main reason for this low percentage was probable overcrowding of the seed in the trays. The reason for not correcting this low germination was that the maize was being "sprouted" according to instructions issued by the firm* supplying the cabinet, and consistent adherence to these instructions was regarded as more important than the introduction of changes calculated to improve the percentage germination. Eight pounds of maize, weighed in the dry condition, was spread in each sprouting tray (area 660 sq. in.); after "sprouting" for 10 days the weight of the product varied from 14 to 22 lb., the increase in weight being chiefly water and some salts from the nutrient solution.

Records were kept of (*a*) the live weight and the food intake of the cows, (*b*) the yields, and (*c*) the fat and solids-not-fat content of the milk, while (*d*) the nutritive value of the milk was also studied in detail.

RESULTS

(*a*) Live weight of cows

The cows were weighed on two or three successive days at the following stages of the experiment: On 2, 4 and 5 January, at the end of the preliminary control period; on 1 and 2 March, after 8 weeks' experimental feeding; on 23, 24 and 25 March, after 11 weeks' experimental feeding; and on 19, 20 and 21 April, during the fourth week of the second control period. The mean weight of each cow at each weighing time and the group averages are shown in Table IV.

The largest increases in weight were shown by the artificially dried grass group, and satisfactory increases were shown by the mangel and the kale groups. On the other hand, every cow in the sprouted maize group lost weight during the first 8 weeks of the experimental period. The probable reason

* Sprout, Ltd., 14–17 Holborn Viaduct, London, E.C.1.

Table IV. *Live weights of cows*

	At end of preliminary control period lb.	After 8 weeks' experimental feeding lb.	After 11 weeks' experimental feeding lb.	Fourth week of the second control period lb.
Winsome 13	1097	1148	1159	1164
Welcome 7	1336	1310	1309	1300
Doris 3	1162	1180	1184	1185
Mean of 3 cows on mangel supplement	1198	1213	1217	1216
Increment	—	+15	+19	+18
Lottie 10	1051	1065	1083	1096
Peach 9	1503	1460	1522	1464
Clara 4	1289	1321	1335	1341
Mean of 3 cows on kale supplement	1281	1282	1313	1300
Increment	—	+ 1	+32	+19
Puppet	1002	1046	1045	1068
Campion 2	1193	1240	1261	1199
Clara 3	1276	1308	1323	1353
Mean of 3 cows on dried grass supplement	1157	1198	1210	1207
Increment	—	+41	+53	+50
Winsome 12	1210	1137	1211	1232
Flora 31	1161	1102	1170	1206
Lena 4	1219	1190	1201	1215
Mean of 3 cows on sprouted maize supplement	1197	1143	1194	1218
Increment	—	-54	- 3	+21

for this decrease was that the sprouted maize used was less palatable than usual, owing to makeshift arrangements for its production through delay in delivery of the complete plant. When improvements had been made in the quality of sprouted maize by better and more consistent methods of growing, the live-weight gains made by the cows compared satisfactorily with those fed other supplements.

(*b*) Milk yields

The milk yields of each animal week by week throughout the experiment are given in Table V. In order to simplify the interpretation of the milk yields they are expressed as percentages in Table VI. The first part of this table shows the relative milk yield of each group of cows during each of the four periods of the experiment, the preliminary control period being expressed as 100. The first 2 weeks of the experimental period and the first week of the second control period were omitted to avoid change-over effects. The last two lines of the table provide a simple comparison between each fodder supplement by expressing the weekly milk yields during the preliminary and second control periods as 100 and the milk yields during the experimental period as a proportional figure. Kale (107·4) and artificially dried grass (107·1) gave the highest comparative yields, while the sprouted maize group (104·3) and mangels

Table V. *Weekly milk yields of cows (lb.)*

Name of cow	Food supplement	No. of days from calving to 13 Dec.	Preliminary control period			Experimental period											Second contro period						Grazing period	
			Week ending:																					
			20 Dec.	27 Dec.	3 Jan.	10 Jan.	17 Jan.	24 Jan.	31 Jan.	7 Feb.	14 Feb.	21 Feb.	28 Feb.	7 Mar.	14 Mar.	21 Mar.	28 Mar.	4 Apr.	11 Apr.	18 Apr.	25 Apr.	2 May	9 May	16 May
Winsome 13	Mangels	17	168	160½	188½	186½	177½	173	177	171	166½	165	156½	160½	158½	154¼	153½	152½	154½	157½	160	156½	177½	184¼
Welcome 7	,,	14	314½	320½	326½	347½	324½	324	325½	327	312½	302½	289½	278½	272	277	262¼	246	244	244	241	245½	240½	238½
Doris 3	,,	34	243½	261	260	251½	245½	241	244½	242¼	233¼	233½	229¼	214	219¾	211¼	209½	207¼	213¼	214¼	214	213	216	227¾
	Total		726	742	775	785¾	747½	738	747¼	740¼	712½	701¼	675¼	653¼	650¼	643	625½	605½	611¾	615½	615	615	634½	650½
Lottie 10	Kale	12	140¼	152	156½	154¼	157	156¾	156¾	154¼	153	150¾	149	144½	144½	145¼	143½	135¼	137¾	138¼	137½	134¼	150½	162
Peach 9	,,	17	206¾	212¾	217¼	215¼	218	221	219¼	223	211½	210	205¾	201½	195	194½	186¼	172½	171¾	179	174¼	173¼	194¼	204¾
Clara 4	,,	9	249¾	258½	258¾	267¼	249¾	246	241¾	232¾	224¼	196½	180¾	177¾	171	155¼	146¼	142¼	120½	117¼	115	103	117¾	120
	Total		596¾	623¼	632½	636¾	624¾	623¾	617¾	610	588¾	557¼	535½	523¾	510½	495	476	450	430	434½	426¾	410½	462¼	486¾
Puppet	Artificially dried grass	80	139¼	141¼	145½	143¾	156	160¼	160	152¼	151½	152¼	149½	131½	136¾	137	134½	128	135	137	141	132¾	140	156
Campion 2	,,	19	245¼	269¾	276¾	285¾	297¼	291½	291½	292¼	288½	279½	264¼	261	253	252¾	256¾	236	251¾	261¾	257	259½	250¾	232¼
Clara 3	,,	81	149½	154¼	153¼	134	137	130¼	127¾	119¾	115¾	113¾	108½	103	97½	92¾	85½	75	66¼	66½	35¾	22¼	10¾	*5¾
	Total		534	565¼	575½	563½	590¼	582	579¼	564¼	555¾	545½	522¼	495½	487¼	482¼	476¾	439	453¼	465¼	433¾	414½	401½	394
Winsome 12	Sprouted maize	89	152½	157¼	170½	176¼	177¼	167¾	161½	169	172¼	173	166¾	151	161½	160½	157	154¾	146¼	150¼	121½	129½	150	163½
Flora 31	,,	11	265½	276½	283¼	284½	288¼	298½	279¾	304½	290¾	281	276¾	271½	262¾	257¾	258¾	247¾	249¾	258	259¼	256½	279¼	290½
Lena 4	,,	47	126	130¼	129½	119	122	121¼	109¾	122½	110¼	108¼	94	86½	86	88½	84¼	83½	80¼	79½	64¼	58¾	64	63
	Total		544	564	583¼	579¾	587½	587½	551	595¾	573¼	562¼	537½	508¾	510¼	506¾	500	486	476¼	487¾	445¼	444¾	493½	517

* Nearly dry, milked once daily.

(102·3) were third and fourth respectively. The differences in milk-yield response of the various foods do not approach statistical significance, and it can only be concluded that if any difference exists it would be necessary to use a much larger number of experimental animals to measure it.

Table VI. *Comparative milk yields*

Periods	Mangels group (controls) (3 cows)	Kale group (3 cows)	Artificially dried grass group (3 cows)	Sprouted maize group (3 cows)
Preliminary control (3 weeks)	100	100	100	100
Experimental (9 weeks)	93·1	91·1	95·8	95·5
Second control (5 weeks)	81·9	69·7	79·0	83·0
Grazing (2 weeks)	85·9	76·8	71·3	89·6
Average yield during preliminary and second control periods	100	100	100	100
Experimental period	102·3	107·4	107·1	104·3

(*c*) Chemical composition of milk

The fat percentage of the milk of each cow was measured on three consecutive days each month. One of these monthly tests was made during the preliminary control period, three during the experimental period, one during the second control period and one during the grazing period.

The non-fatty solids content of the milk of each cow was determined on one day each month. These tests were made as a check on the quality of the milk produced by the cows and were not regarded as a critical test of the effect of the various foods on the fat and solids-not-fat content of milk. The results, however, are of interest, and the group averages are shown in Table VII.

Table VII. *Fat and non-fatty solids content of the milk*

Fodder supplement of		Preliminary control period %	Experimental period %	Second control period %	Grazing period %	Relative results: During preliminary and second control period	Relative results: During experimental period
Mangels:	Fat	4·25	3·53	3·03	3·43	100	103
	S.N.F.	8·67	8·69	8·71	8·78	100	100
Kale:	Fat	4·10	3·55	3·49	4·05	100	107
	S.N.F.	8·78	8·73	8·80	8·84	100	99
Artificially dried grass:	Fat	3·75	3·47	3·24	*3·50	100	101
	S.N.F.	8·56	8·61	8·63	*8·50	100	100
Sprouted maize:	Fat	3·63	3·35	3·04	3·39	100	100
	S.N.F.	8·96	8·85	8·84	8·67	100	99

* Two cows only. Clara 3 omitted as nearly dry.

All four groups of cows show relatively high fat percentages during the preliminary control period; this was undoubtedly due to the fact that most of the cows were recently calved. A simple measure of the effect of the foods on the fat and solids-not-fat content of the milk is shown by the two right-hand

columns of Table VII, where the mean of the preliminary and second control periods are expressed as 100 and the experimental periods as relative figures.

It will be seen that the differences between the various groups are negligible; they do not approach statistical significance, and it may be concluded that the foods had little, if any, influence on the fat and solids-not-fat content of the milk.

(*d*) The nutritive value of the experimental milks

The nutritive properties of the milks produced by the four groups of cows were measured in biological experiments on rats and by physical and chemical tests. The following factors were studied:

(1) Vitamin A and carotene (physical and chemical measurements).
(2) The vitamin B complex (biologically).
(3) The vitamin B_2 complex (biologically).
(4) Vitamin C (chemically).
(5) The total nutritive value of the milks (biologically).

In view of the high correlation which is known to exist between the carotene in the food of the cow and the vitamin A and carotene in her milk, it was expected that in this respect definite differences would be found between the experimental groups.

According to the work of Gunderson & Steenbock(4) and to the more recent findings reviewed by Kon(1, 2) no such differences should be found with the vitamin B complex. There is no unanimity of opinion regarding the connexion between the "vitamin B_2" content of fodder and of milk (Kon(1, 2)), and it was hoped that the estimation undertaken in the present study might throw some light on the subject. It seemed unlikely that the level of vitamin C in milk would alter with changes in the food of the cow (Kon(2, 3)), but this point warranted confirmation.

The experiments on the total nutritive value of the milks or rather on their growth-promoting properties when fed (supplemented with minerals) as an exclusive diet to rats were carried out in an attempt to investigate the passage from fodder to milk of the less well-defined essential dietary factors mentioned on p. 273. The present authors had previously been unable to confirm the findings of the Wisconsin workers concerning the seasonal fluctuation of these factors in milk(2, 3). They had, however, used a different experimental technique. In the present study both techniques were used simultaneously in an effort to resolve the differences.

The experiments described under (1) extended throughout the full duration of the tests, but the remaining experiments were carried out only during the experimental feeding period (i.e. period 2). More reliable results would have been obtained if the various factors had been estimated also in the first control period, so as to allow for individual differences between the several groups of cows which were not due to treatment. For technical reasons this was

unfortunately not possible, and this limitation should be borne in mind in interpreting the results.

Where data were investigated statistically the methods described by White were used (5, 6).

(1) *Vitamin A and carotene (in collaboration with A. E. Gillam, University of Manchester)*

Vitamin A and carotene estimations were carried out on butter from all four groups of cows. A sample of butter was made from the milk of each group on the day before experimental feeding started; subsequently throughout the experiment aliquot samples of the morning and evening milkings were collected daily from all groups of cows until they had been on grass for 17 days. The samples were pooled within each group and churned twice a week. The bi-weekly samples of butter were combined and rendered each week for analysis. At the beginning of period 3 samples from the artificially dried grass and kale groups were analysed every 2 days for the first week and subsequently twice weekly. Butter from the other two groups was analysed twice in the first week and once each week subsequently.

Additional samples of butter from the artificially dried grass and control groups were taken at a later stage of grazing. Lovibond Tintometer tests (K. M. H. and S. K. K.) were carried out on all samples. In addition, the vitamin A and carotene content of certain representative samples was estimated spectrophotometrically (A. E. G.). Both methods of analysis have been fully described elsewhere (Gillam *et al.* (7)).

Figs. 1 and 2 show graphically the vitamin A and carotene contents of the butters from all four groups of cows, expressed in Moore blue and yellow units.

All groups started approximately level, but a rapid differentiation took place immediately after the experimental feeding was begun. Two types of vitamin A and carotene curves will be noted: one for the control and sprouted maize groups and another for dried grass and kale. The curves for the first two are very similar. Though sprouted maize produced at first a slight and transient rise in the vitamin A content of the butter while the values for the controls were stationary, a fall rapidly set in and there was little to chose between the two groups. With carotene the agreement was still more marked.

The vitamin A curves, which rise sharply for the dried grass and kale groups, demonstrate the marked effect of these fodders. A peak is reached in both curves in about 3 weeks and is followed by a gradual fall. Higher values were reached by the dried grass butters, but the starting point for this group was also slightly higher. After reaching the peak, however, this group fell off more rapidly.

The carotene curves show the superiority of the dried grass over kale. A peak is noticeable only for the former and occurs later than the vitamin A peaks. The rise in carotene of these two groups is, however, just as sharp as

for vitamin A, the initial values being more than doubled. The rise is less apparent because of the arbitrary nature of the scale for blue and yellow units.

As would be expected, the change from experimental feeding to the control ration had very little effect on the vitamin A and carotene level of the sprouted maize group. The plateau which followed the steady decline in vitamin A values was reached on the experimental diet before the change. After some 3 weeks a further decline set in, and the curve for vitamin A ran parallel to that for the experimental period.

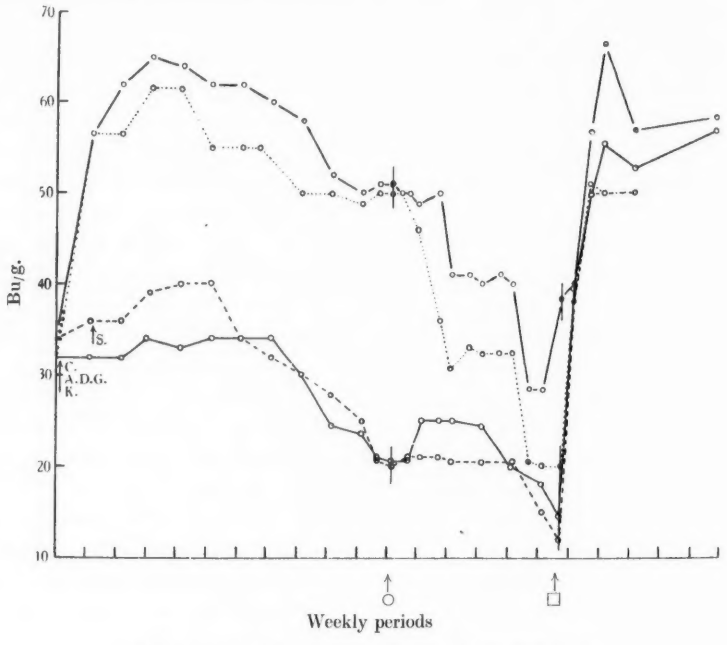

Fig. 1. Vitamin A content of butters (Moore blue units).

↑ Experimental feeding started.

↑ Experimental feeding finished. All groups on control ration 3.30 p.m.

↑ All cows put out to grass after morning milking.

○———○ Control. ○ ——— ○ Artificially dried grass.

○ - - - - - - ○ Sprouted maize. ○ - - - - - - - ○ Kale.

The dried grass and kale groups responded much more obviously to the change in feeding, and the decline in vitamin A values which had already started on the experimental feeding became definitely steeper. With the dried grass group the carotene curve is obscured by the trough just after the change of diet and the unspecific rise immediately after, but, on the whole, the slope of the curve is hardly altered. On the other hand, the rate of decline of the kale group became more rapid.

There was, of course, no change in diet in the control group itself. An unspecific rise in vitamin A occurred at the beginning of the 3rd period, but this was quickly followed by a further decline.

The reaction following turning out to grass was very similar in all four groups and was characterized by an immediate rise in both carotene and vitamin A levels. Peak values for vitamin A were reached within 10 days, but the carotene figures continued to increase for 5 weeks in the dried grass and control groups. The behaviour of the remaining two groups was not studied for so long a period, but $2\frac{1}{2}$ weeks after grazing started there was still no evidence of a plateau in the curves.

On pasture the concentration of vitamin A in the sprouted maize butter, which was the lowest of the four groups just before grazing, rose from 12

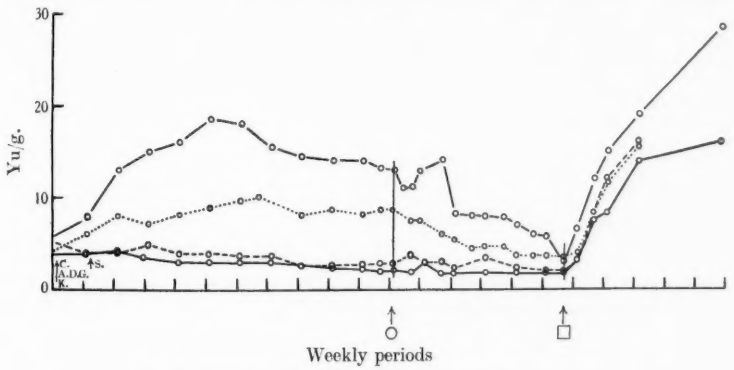

Fig. 2. Carotene content of butters (Moore yellow units).

↑ Experimental feeding started.

↑ ○ Experimental feeding finished. All groups on control ration 3.30 p.m.

↑ □ All cows put out to grass after morning milking.

○———○ Control). ○ ——— ○ Artificially dried grass.

○ - - - - - - ○ Sprouted maize. ○ - - - - - - - ○ Kale.

to 50 units per gram, while the dried grass butter, richest at the beginning, increased from 39 to 67. The kale butter, though richer at the start than the control butter, did not reach such high values as the latter.

When the effect on carotene is considered the curves show that its concentration increased ninefold in both the dried grass and the control butters, which were respectively highest and lowest in carotene at the start. The highest value reached by the control group was, however, barely two-thirds of the corresponding value of the dried grass cows. In view of the smallness of the experimental groups and also because at the very outset of the experiment the dried grass butter contained more carotene than the control butter, it is impossible to say whether the final difference was due to some idiosyncrasy of the cows or was related to the previous experimental feeding.

An interesting point emerges in relation to the time necessary for the carotene and vitamin A of butter to reach equilibrium after a change has been made in the diet of the cow which affects the level of carotene intake. Loy *et al.* (8), believe that when both diets contain some carotene the equilibrium is reached in about 11 days. They suggest further that when the change is made from a satisfactory diet to one devoid of carotene the initial decline in vitamin A and its precursor would be rapid at first and become gradual later.

The present observations support neither of these views. Fig. 2 shows, for example, that with the dried grass group the repletion curves for carotene, whether the cows received the dried grass or were on pasture, rose steadily for some 5 weeks. The depletion curve for the same group after the change from experimental to control feeding declined smoothly after an initial unspecific increase. Further, in a short-period experiment, it would be difficult to determine when equilibrium was reached with vitamin A. The kale and dried grass groups showed on the experimental diets, for example, a steady rise for 3 weeks followed by a decline for another 6 or 7 weeks.

Unspecific changes in the vitamin A or carotene level of the butter may occur and cause difficulty in short feeding trials. Thus if the effect on the carotene level of the change from the dried grass to the control ration were to be measured for only a short time a completely fallacious conclusion might be drawn as a result of the unexpected temporary rise in carotene values.

Table VIII gives a comparison of Lovibond Tintometer and spectrophotometric estimations of carotene and vitamin A on selected samples of butter. This table shows that there is, on the whole, good agreement between the two methods and that the routine Lovibond estimations on which Figs. 1 and 2 are based may be accepted with confidence.

In Table IX the carotene content of the various fodders used in the experiment is set out. The carotene was estimated by the method of Ferguson & Bishop (9). The curve used for relating depth of colour to concentration of carotene was based on data obtained in this laboratory.

The sample of kale consisted of about 56 % leaves and 44 % stalks. The hard wooden ends of the stalks which would normally be rejected by the cows were not analysed. A rough calculation shows that on the dry basis the kale as taken by the cows would contain 11·4 mg. carotene/100 g., i.e. less than an equal weight of the artificially dried grass. The carotene content of the artificially dried grass was lower than is usual for this product. The sprouting, if anything, lowered the pigment value of maize. Mangels and sugar-beet pulp were not analysed for carotene, as they are known to contain only negligible quantities.

Table X gives carotene balances for selected periods. The balances of carotene and vitamin A shown in this table were calculated for specific days during the course of the experiment, from milk and fat yields. The quantities of foods are as given in Table II, with the exception of kale, which was calculated at

Table VIII. *The vitamin A and carotene content as determined spectrophotometrically and by Lovibond Tintometer, of butter samples from the four groups of experimental cows*

	Control		Artificially dried grass		Sprouted maize		Kale	
	Spectrophotometrically	Lovibond Tintometer	Spectrophotometrically	Lovibond Tintometer	Spectrophotometrically	Lovibond Tintometer	Spectrophotometrically	Lovibond Tintometer
	Vitamin A							
	mg. vit. A/100 g. fat*	Moore blue units/g. fat	mg. vit. A/100 g. fat*	Moore blue units/g. fat	mg. vit. A/100 g. fat*	Moore blue units/g. fat	mg. vit. A/100 g. fat*	Moore blue units/g. fat
Pre-experimental feeding	0·86	32·0	0·93	34·0	0·86	34·0	0·92	32·0
Experimental feeding: 2nd week	0·75	32·0	1·07	62·0	0·80	36·0	1·01	56·5
5th and 6th weeks	0·90	34·0	1·24	62·0	0·95	37·0	1·04	55·0
last 4 days	0·59	20·5	1·11	51·2	—	20·0	0·85	50·0
Second control period: 2nd week	0·81	25·0	0·85	50·0	—	21·0	0·79	36·0
last 4 days	0·39	14·5	<1·14	38·6	<0·55	12·0	0·61	20·0
After 16 days on grass	1·27	52·9	1·57	57·1	1·04	50·0	1·30	50·0
	Carotene							
	mg. carotene/100 g. fat†	Moore yellow units/g. fat	mg. carotene/100 g. fat†	Moore yellow units/g. fat	mg. carotene/100 g. fat†	Moore yellow units/g. fat	mg. carotene/100 g. fat†	Moore yellow units/g. fat
Pre-experimental feeding	0·145	3·8	0·207	5·8	0·180	5·1	0·173	4·7
Experimental feeding: 2nd week	0·120	4·0	0·365	12·9	0·130	3·9	0·240	8·0
5th and 6th weeks	0·137	3·2	0·530	18·2	0·175	3·8	0·300	9·2
last 4 days	0·090	2·2	0·540	12·9	<0·190	2·9	0·330	8·7
Second control period: 2nd week	0·090	1·8	0·420	14·2	0·140	3·0	0·300	6·0
last 4 days	0·044	1·7	0·280	3·0	0·054	2·1	0·120	3·6
After 16 days on grass	0·570	13·8	0·790	19·1	0·590	16·0	0·610	15·6

* Based on the assumption that vitamin A itself has an intensity of absorption at 328 mμ $E^{1\%}_{1cm.}$ = 1350 (in $CHCl_3$). (Gillam & El Ridi (10).)

† These figures are calculated on the basis of $E^{1\%}_{1cm.}$ = 2200 for pure carotene in $CHCl_3$ at 463 mμ.

Table IX. *Moisture and carotene contents of the main constituents of the experimental rations*

	% moisture	Mg. carotene/100 g. Moist	Dry
Hay	19·66	0·39	0·48
Maize (flaked)	17·17	0·91	1·10
Maize (before sprouting)	14·48	0·89	1·05
Cubes	15·87	0·26	0·30
Artificially dried grass	13·04	12·89	14·82
Sprouted maize	62·26	0·32	0·85
Kale: Leaves	83·78	3·11	19·18
Stalks	80·04	0·29	1·45

Table X. *Intake of carotene and its output as vitamin A and carotene, per cow per day*

Period	Dietary group	Intake of carotene mg.	Yield of* butterfat g.	Output in milk: Carotene mg.	Vitamin A mg.
Preliminary control	Control (mangels)	40·9	716	1·00	5·87
	Artificially dried grass	36·6	408	0·86	3·55
	Sprouted maize	37·4	413	0·74	3·59
	Kale	38·9	444	0·75	3·64
Experimental (2nd week)	Control (mangels)	40·1	539	0·76	4·42
	Artificially dried grass	846·2	444	2·04	7·06
	Sprouted maize	37·4	422	0·59	3·88
	Kale	393·2	458	1·33	6·64
Experimental (6th week)	Control (mangels)	40·1	598	0·66	5·20
	Artificially dried grass	845·8	403	2·62	6·41
	Sprouted maize	42·4	394	0·55	3·74
	Kale	392·8	417	1·38	5·88
Experimental† (10th week)	Control (mangels)	39·7	471	0·38	2·50
	Artificially dried grass	845·8	363	1·67	4·76
	Sprouted maize	42·4	358	0·36	1·83
	Kale	392·0	381	1·18	4·88
Second control (2nd week)	Control (mangels)	38·2	466	0·28	2·98
	Artificially dried grass	35·4	317	1·62	4·06
	Sprouted maize	35·4	319	0·35	1·72
	Kale	35·0	338	0·71	3·11
Second control (6th week)	Control (mangels)	37·0	441	0·26	1·63
	Artificially dried grass	35·4	292	0·32	2·89
	Sprouted maize	33·9	294	0·23	0·91
	Kale	33·6	306	0·40	1·56
Grazing (17th day)	Control (mangels)	—	471	2·31	6·46
	Artificially dried grass	—	263	1·79	3·84
	Sprouted maize	—	358	2·04	4·58
	Kale	—	399	2·23	5·11

* The yields of fat for the above table were calculated from individual cow tests taken twice daily on three consecutive days, while the carotene and Vitamin A values were estimated from bulked milks. The days of sampling for the two determinations were not always identical but corresponded closely.

† The yield of fat applies to March 16–18 and the carotene and vitamin values to March 22–25, i.e. last 4 days of Table VIII.

42 lb. per cow per day instead of 46 lb.; the difference represents an estimate of the woody stems of kale refused by the cows.

The output of carotene and vitamin A in butter (milk) was calculated from Lovibond Tintometer readings given in Table VIII using the factors: Vitamin A (mg./100 g. fat) = Moore blue units/g. fat $\times \frac{1}{39}$, and carotene (mg./100 g. fat) = Moore yellow units/g. fat $\times \frac{1}{28}$. These factors are provisional and are taken from unpublished data obtained by us in collaboration with Dr A. E. Gillam of Manchester University. These figures are used in preference to the spectrophotometric estimations which are incomplete.

(2) *Vitamin B complex and vitamin B_2 complex*

In this investigation one series of experiments dealt with the value of the different milks in supplementing a diet deficient in all the components of the vitamin B complex. In another series a Kinnersley & Peters(11) concentrate from baker's yeast (prepared by the alternative technique which avoids the use of mercury, up to the stage of extraction with $N/10$ HCl from charcoal) supplemented the deficient ration. In addition to vitamin B_1 such crude extracts most probably contain vitamin B_6 and possibly other members of the complex (Chick *et al.*(12)). Whether, therefore, in these experiments lactoflavin alone was measured or a "vitamin B_2 complex" is a matter of conjecture.

Experimental.

In both series of experiments the rats were prepared for the tests by the routine method employed in this laboratory (Kon(13)). Only female rats were used. The experiments were confined to milks from the control, dried grass and sprouted maize groups.

(*a*) *Vitamin B complex.* Eight sets of three and one pair of litter-mate female rats, when "run-out" were allocated by lot to control, dried grass or sprouted maize milk. The animals were kept in cages in groups of three or two and were given 8 ml. of the appropriate milk daily (except on Sundays) in special individual feeding cages. The rats were weighed twice weekly and the experiment lasted for 8 weeks.

Average growth curves for the three groups of animals are shown in Fig. 3. It will be seen that the differences between the groups are very small. Table XI gives the individual figures for the gains in weight of the rats after 4 and 8 weeks on the experimental milks.

These figures show that the gains in weight of the three groups are very similar and that the differences can obviously have no statistical significance. Two animals on the sprouted maize milk, rats 6067 and 6151, developed symptoms of beri-beri after having received the milk for 6 and 7 weeks respectively, but both survived until the end of the experiment. The symptoms were more marked in rat 6067.

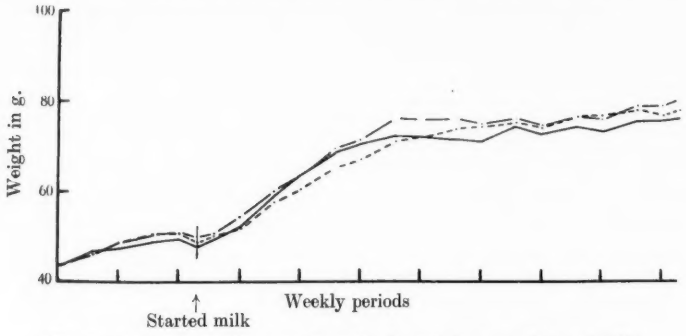

Fig. 3. Average growth curves for animals receiving experimental milks as sole source of the vitamin B complex.

——— Sprouted maize. ·———· Artificially dried grass. ·-------· Control.

Table XI. *Gains in weight after 4 and 8 weeks of rats receiving control, dried grass or sprouted maize milks as the sole source of the vitamin B complex*

	Control			Artificially dried grass			Sprouted maize		
		Gain in weight (g.)			Gain in weight (g.)			Gain in weight (g.)	
Litter no.	Rat no.	4 weeks	8 weeks	Rat no.	4 weeks	8 weeks	Rat no.	4 weeks	8 weeks
I	6029	33	38	6028	31	20	6025	13	19
II	6043	19	24	6040	31	28	6042	32	37
III	6058	23	27	6059	30	33	6057	23	31
IV	6064	29	40	6066	24	26	6067*	29	19
V	6080	33	40	6082	18	25	6083	33	39
VI	6152	11	19	6150	4	3	6151*	3	−9
VII	6160	31	31	6158	26	41	6161	34	47
VIII	6171	16	16	6166	38	51	6167	30	48
IX	6184	28	30	6181	40	50	—	—	—
Mean		24·8	29·4		26·9	30·8		—	—
Mean omitting litter IX		24·4	29·4		25·3	28·4		24·6	28·9

* Symptoms of beri-beri.

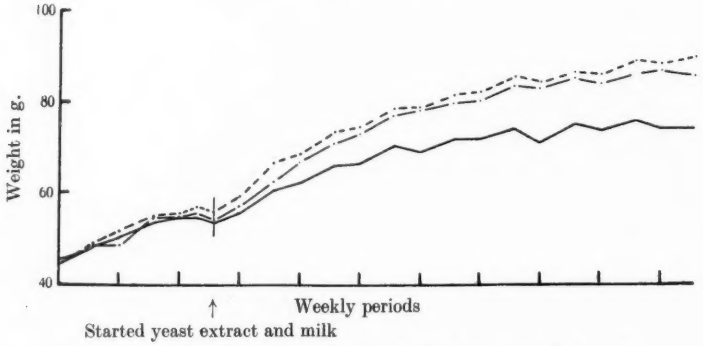

Fig. 4. Average growth curves for animals receiving experimental milks as sole source of the vitamin B_2 complex.

——— Sprouted maize. ·———· Artificially dried grass. ·-------· Control.

(*b*) *Vitamin B_2 complex.* Eight sets of three litter-mate female rats, when "run-out", were allocated to the experimental milks by lot. The rats were kept in cages in groups of four and received daily (except on Sundays) 4 ml. of the appropriate milk together with 0·75 ml. of the Kinnersley & Peters[11] yeast extract equivalent to 1·5 g. dry yeast. The animals were weighed twice a week and the experiment lasted for 8 weeks after the commencement of milk feeding.

Fig. 4 shows the average growth curves for the rats receiving the three experimental milks. Table XII gives the individual gains in weight of the animals after they had received the milks for 4 and 8 weeks.

Table XII. *Gains in weight after 4 and 8 weeks of rats receiving control, dried grass and sprouted maize milks as the sole source of the vitamin B_2 complex*

	Control			Artificially dried grass			Sprouted maize		
		Gain in weight (g.)			Gain in weight (g.)			Gain in weight (g.)	
Litter no.	Rat no.	4 weeks	8 weeks	Rat no.	4 weeks	8 weeks	Rat no.	4 weeks	8 weeks
I	6030	26	39	6026	38	57	6027	20	29
II	6049	14	21	6050	37	52	6048	16	19
III	6072	17	35	6073	35	49	6074	25	26
IV	6088	27	34	6089	25	26	6090	17	21
V	6098	23	25	6096	7	6	6097	25	24
VI	6199	35	40	6200	17	14	6198	12	12
VII	6222	31	35	6223	19	20	6220	10	17
VIII	6228	35	46	6230	31	35	6229	25	23
Mean		26·0	34·4		26·1	32·4		18·8	21·4

Fig. 4 shows that the growth of the rats receiving the sprouted maize milk was inferior to that of the other two groups. This inferiority became more marked as the experiment progressed. The growth of the rats receiving the control milk was slightly better than that of the dried grass group. The same is evident from Table XII where the average gains in weight at 4 and 8 weeks are given. Table XIII shows that, as would be expected, the differences in gain in weight of the control and dried grass groups are not statistically significant. It also shows that at the end of the experiment the gain in weight of the sprouted maize group was significantly less than that of the control group, the odds being 161 to 1 against the difference being due to chance. The differences between these two groups after 4 weeks lack statistical significance, with odds of 9 to 1. Similarly, the differences after 4 and 8 weeks between the dried grass and sprouted maize group, although suggestive, are not statistically significant.

It appears from these results that the sprouted maize milk was a less rich source of the vitamin B_2 complex than the other milks. It is not possible to say whether this difference was a result of the feeding or whether it was due to differences in the cows themselves. Nevertheless, when compared with the results in the previous series of experiments these findings suggest a greater variability in the vitamin B_2 complex of milk than in the vitamin B complex.

Table XIII. *Statistical treatment of the differences in gains in weight after 4 and 8 weeks of rats receiving control, dried grass and sprouted maize milks as the sole source of the vitamin B_2 complex*

	Gain after 4 weeks	Gain after 8 weeks
Mean for control (g.)	26·0	34·4
Mean for dried grass (g.)	26·1	32·4
Difference: control – dried grass (g.)	− 0·1	+ 2·0
Standard error of mean	± 5·58	± 7·19
*P**	1 : 1	1 : 1
	Not significant	Not significant
Mean for dried grass (g.)	26·1	32·4
Mean for sprouted maize (g.)	18·8	21·4
Difference: dried grass – sprouted maize (g.)	+ 7·3	+11·0
Standard error of mean	± 4·14	± 5·88
*P**	1 : 8	1 : 10
	Not significant	Not significant
Mean for control (g.)	26·0	34·4
Mean for sprouted maize (g.)	18·8	21·4
Difference: control – sprouted maize (g.)	+ 7·2	+13·0
Standard error of mean	± 3·92	± 3·38
*P**	1 : 9	1 : 161
	Not significant	Significant

* P = probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

(3) *Vitamin C*

The vitamin C content of the experimental milks was estimated on a number of blended samples used for the feeding tests. The dichlorophenolindophenol titration as described by Kon & Watson(14) was used. Table XIV gives the vitamin C content (total and reduced) of eight samples of the control, dried grass and sprouted maize milks and of seven samples of the kale milk. This last milk was not used for feeding tests, but small samples from morning and evening milkings were kept and mixed under conditions similar to those used for the other milks. Table XV shows that the differences between the means were not great. The total and reduced vitamin C contents of the sprouted maize milk were, however, significantly higher than those of the dried grass and control milks and the difference in reduced but not in total, ascorbic acid between the sprouted maize and kale milks was significant. The kale milk was also found to be significantly richer in the total vitamin C (though not in reduced) than the dried grass milk. The remaining differences are probably due to chance. It is not possible to say whether the slight superiority of the sprouted maize milk is due to the feeding or to inherent differences between the cows.

It is noticeable that the values obtained were invariably higher than those quoted for "sale" milk by Kon & Watson(15). It is possible that this is due to the fact that the "sale" milk had been passed over a cooler while the milks used in the present work had not been so treated.

Table XIV. *The vitamin C content of the milks from the four groups of experimental cows*

Date	Control milk mg. vit C/100 ml.		Artificially dried grass milk mg. vit. C/100 ml.		Sprouted maize milk mg. vit. C/100 ml.		Kale milk mg. vit. C/100 ml.	
	Total (reversibly oxidized and reduced)	Reduced	Total (reversibly oxidized and reduced)	Reduced	Total (reversibly oxidized and reduced)	Reduced	Total (reversibly oxidized and reduced)	Reduced
15. ii. 37	2·518	2·077	2·267	1·951	2·623	2·245	—	—
17. ii. 37	2·518	2·014	2·434	2·161	2·602	2·224	2·685	2·140
22. ii. 37	2·383	2·043	2·509	2·043	2·797	2·438	2·707	2·313
24. ii. 37	2·545	1·936	2·545	2·007	2·797	2·258	2·617	1·936
2. iii. 37	2·632	2·236	2·560	2·254	2·740	2·397	2·668	2·254
3. iii. 37	2·614	2·055	2·651	2·254	2·849	2·380	2·704	2·127
8. iii. 37	2·733	2·291	2·715	2·346	2·844	2·401	2·936	2·327
10. iii. 37	2·973	2·291	2·826	2·272	3·029	2·456	2·882	2·327
Mean omitting 15. ii. 37	2·628	2·124	2·606	2·191	2·808	2·365	2·743	2·203
Mean (all)	2·614	2·118	2·564	2·161	2·785	2·350	—	—

Table XV. *Statistical treatment of the differences in the vitamin C content (total and reduced) of the milks from the four groups of experimental cows*

	mg. vit. C/100 ml.			Mg. vit. C/100 ml.	
	Total	Reduced		Total	Reduced
Mean: Control	2·614	2·118	Mean: Kale	2·743	2·203
Dried grass	2·564	2·161	Control	2·628	2·124
Difference: control – dried grass	+0·050	−0·043	Difference: kale – control	+0·115	+0·079
Standard error of mean	±0·041	±0·036	Standard error of mean	±0·050	±0·035
*P**	1 : 4 Not significant	1 : 4 Not significant	*P**	1 : 16 Not significant	1 : 15 Not significant
Mean: Dried grass	2·564	2·161	Mean: Control	2·614	2·118
Sprouted maize	2·785	2·350	Sprouted maize	2·785	2·350
Difference: dried grass – sprouted maize	−0·221	−0·189	Difference: control —sprouted maize	−0·171	−0·232
Standard error of mean	±0·025	±0·042	Standard error of mean	±0·043	±0·036
*P**	<1 : 11000 Significant	1 : 250 Significant	*P**	1 : 192 Significant	1 : 1600 Significant
Mean: Sprouted maize	2·808	2·365	Mean: Kale	2·743	2·203
Kale	2·743	2·203	Dried grass	2·606	2·191
Difference: sprouted maize – kale	+0·065	+0·162	Difference: kale – dried grass	+0·137	+0·012
Standard error of mean	±0·042	±0·035	Standard error of mean	±0·032	±0·048
*P**	1 : 6 Not significant	1 : 294 Significant	*P**	1 : 200 Significant	1 : 1 Not significant

* See note to Table XIII.

(4) *Experiments on the growth-promoting value of the milks*

Hart, Elvehjem and their colleagues observed that, when mineralized milk *ad lib.* was fed as a sole diet to rats, alterations in the nature of the milk induced marked differences in growth rate. They found the method very useful in assessing the nutritive value of milk. Similar experiments in which, however, the intakes of the milks were equalized, failed, in our hands, to yield satisfactory results. All these investigations have been reviewed elsewhere (2, 3).

In the present experiments we compared the nutritive effects of the milks produced by the control cows, the cows receiving artificially dried grass and the cows on sprouted maize. We not only used our customary technique but also followed as carefully as possible the methods of the Wisconsin workers.

The two techniques were applied to two types of experiment: in one the milks were supplemented with minerals and fed as the sole diet; in the other, cane sugar was also added to the milks to increase their caloric value and thus cut down the intake of all milk constituents.

Experimental.

(*a*) *The experimental milks.* As in previous work from this laboratory (Henry *et al.* (16)), the evening and morning milkings of each of the three groups of cows were kept separate each day and the fat percentages determined. The milk of each group was then so blended that all three milks had the same fat content. Table XVI gives the average figures for the percentages of fat and total solids in the experimental milks.

Table XVI. *Mean percentage figures for fat and total solids in the experimental milks*

Milk	Control	Artificially dried grass	Sprouted maize
% Fat	3·65	3·65	3·66
% Total solids	12·33	12·14	12·48

On Saturdays a double supply of the milks was obtained and half was stored in the refrigerator for use on Sundays. This milk was brought to room temperature before it was used for feeding.

(*b*) *The experimental animals.* Following the suggestion of Hart (17), all rats used in these experiments were specially prepared as follows. (The method is similar to that described by Kohler *et al.* (18), whose paper appeared after the present experiments were completed, except that these authors separated the young rats from their mothers on the 10th day of lactation.) From the 14th day of lactation onwards all food was removed from the cages containing the litters, and the mothers were removed for 2 hr. morning and evening and allowed access to their diet in separate cages. Any diet adhering to the coats of the mothers was carefully brushed off before returning them to the litter cages. The young rats had access to winter milk and water in their cages.

They were weaned when 21 days old and immediately placed in experi-

mental cages. Only male rats were used. Details of technique will be found elsewhere (16, 19, 20). In all experiments the feeding was continued for 10 weeks. No mineral additions were made on the first day of the experiment.

(*c*) *First experiment. Exclusive feeding of milk supplemented with minerals.* (i) Ad libitum *feeding.* Eight groups of three and two groups of two litter-mate rats from nine litters weighing from 32 to 45 g. were allocated by lot to control, dried grass or sprouted maize milk. Owing to the special treatment the young rats were below our normal weight at weaning. During the first week of the experiment and on all Saturdays and Sundays, the mineral salt mixture was given first thing in the morning mixed with 5 ml. of the appropriate milk. Otherwise the amount of milk mixed with the salt mixture was increased by 5 ml. per week until a level of 25 ml. was reached. When this milk had been consumed the balance of the day's ration was fed. This method of feeding was adopted in order to prevent undue separation of the fat. The milk was fed *ad libitum* and each day an excess over the previous day's consumption of about 15 ml. of milk was offered. The experiment lasted from 9 January to 21 March 1937.

(ii) "*Equalized*" *intake.* Five groups of three and three groups of two litter-mate rats from six litters weighing from 34 to 50 g. were allocated to each of the experimental milks by lot. The milk intake was equalized within groups according to the technique described elsewhere (16, 19). The experiment lasted from 8 January to 22 March 1937.

(*d*) *Second experiment. Feeding of milk containing a definite proportion of dissolved sugar and supplemented with minerals.* (i) Ad libitum *feeding.* Eight groups of three litter-mate rats from seven litters were allocated to the three experimental milks by lot. The salt mixture was given mixed, as usual, with a small amount of the milk and sugar solution, the amount used being gradually increased from 2 to 5 ml. in the course of a fortnight. During the first fortnight 40 g. of castor sugar were added per 100 ml. of milk. By this means the milk intake could be cut down very markedly so as to ensure that the animals were as far as possible depleted of any unknown factor present in milk. Subsequently the experiment proper was started in which 20 g. of castor sugar were added per 100 ml. of milk, yielding 114 ml. of solution. The caloric value per given volume was thus doubled. This type of feeding was continued for 8 weeks. The solution was fed *ad libitum*, about 10 ml. in excess of the previous day's consumption being offered daily. The experiment lasted from 8 January to 22 March 1937.

(ii) "*Equalized*" *intake.* Eight groups of three litter-mate rats from six litters were allocated to the experimental milks by lot. This experiment was similar to that just described except that the milk intake was equalized within groups of three. The experiment lasted from 9 January to 19 March 1937.

At the end of each of these experiments the rats were killed with coal gas and the body length measured (Donaldson (21)).

Results.

(*a*) *The first experiment—exclusive milk feeding.* The results of this experiment are shown in Tables XVII–XX. As litter-mates were not quite evenly distributed between the experimental milks (in certain cases only two litter-mates were available), it was necessary to reject certain animals in litter-mate comparisons between the milks. For this reason two sets of mean figures are given in the tables.

Table XVII, which deals with the *ad libitum* feeding, gives the weights (initial, at 2 weeks, and at 10 weeks), the total gains in weight, the daily gains in weight, the milk intakes and gains per 100 ml. of milk intake for the whole experiment and for its last 8 weeks. It also gives the body lengths of the rats on the three experimental milks.

Table XVIII gives similar figures for the experiment in which the milk intake was "equalized" within groups of three. This table also gives the total amounts of milk left by each rat and the number of days on which the milk was refused. Table XIX gives the statistical treatment of some of the differences shown in Tables XVII and XVIII.

As already mentioned, the feeding of the sprouted maize began 9 days later than that of the artificially dried grass and kale. The sprouted maize group of cows was, therefore, really on the control ration for the first 9 days of the experiment. We felt that when comparing the performance of this group with that of other groups, the first fortnight should be omitted. For this reason, calculations have been made not only for the whole experimental period but also for the last 8 weeks. Tables XVII and XVIII show that even by this method of calculation no differences are disclosed between the milks. The average growth curves of the animals fed the three types of milk *ad libitum* are shown in Fig. 5, while Fig. 6 gives the curves for the animals whose milk intake was "equalized".

It may be stated at once that both these methods of feeding the milks have failed to demonstrate any differences in the nutritive value of the milks from the three groups of cows. Considering first the experiment in which the milk was fed *ad libitum*, we find that the average gain in weight of the group of rats receiving the control milk is 5·7 g. more than that of the dried grass group. Reference to Table XIX shows that this difference is not statistically significant. The difference can undoubtedly be ascribed to the fact that the control rats consumed more milk than their dried grass litter-mates, as the gains per 100 ml. of milk consumed are identical. When the dried grass and sprouted maize milks are compared, it appears that the rats receiving the former gained, on the average, 11·3 g. more than those receiving the latter; the difference is, however, not statistically significant, the probability that the difference was due to chance being 7 : 1. Here, again, the greater gain is due to a higher consumption of milk; the gains per volume of milk being very similar. The slight difference in favour of the control milk over the sprouted maize is not statistically significant. The very slight differences in the body lengths of the three groups of rats are also not significant.

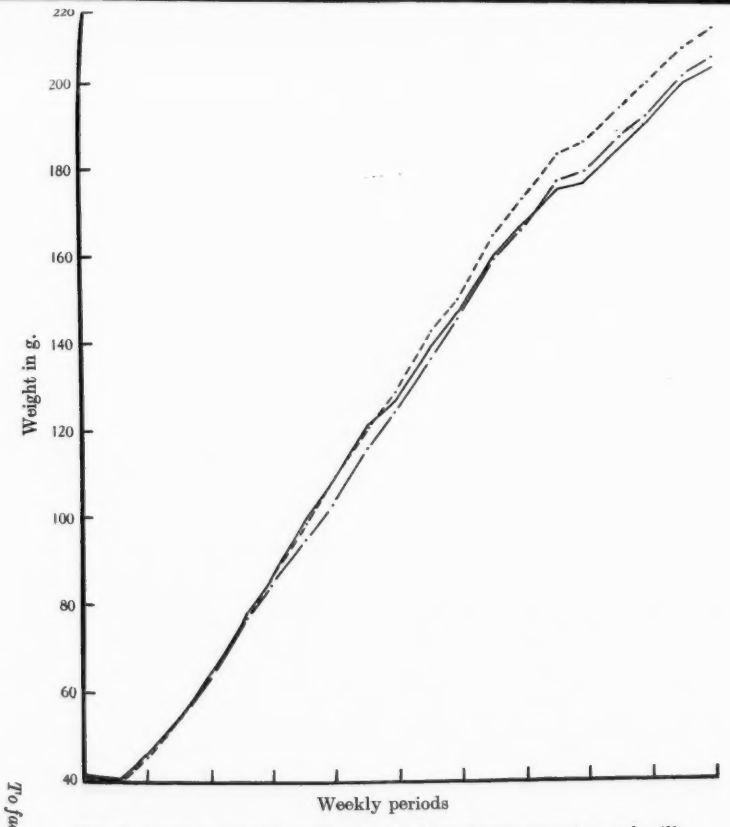

Fig. 5. Average growth curves of animals receiving experimental milks supplemented with minerals, *ad libitum*.

——— Sprouted maize. ·———· Artificially dried grass.
·–·–·–·–· Control.

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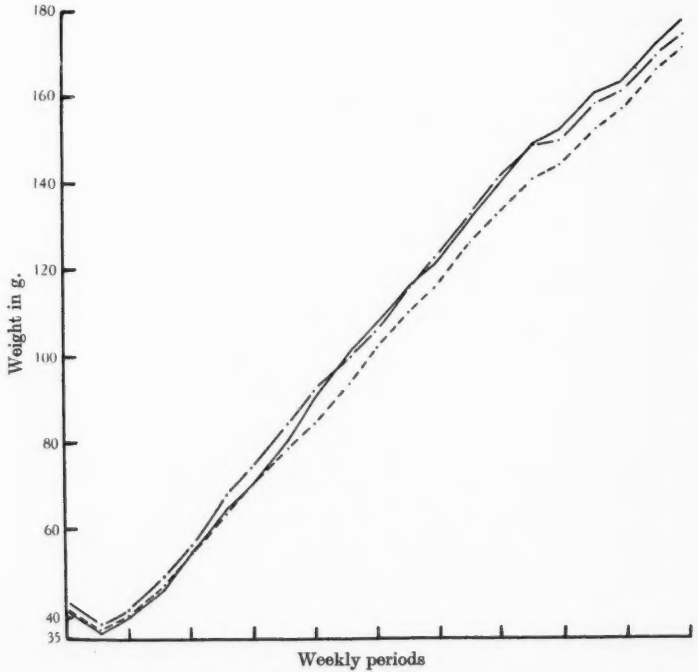

Fig. 6. Average growth curves of animals receiving experimental milks supplemented with minerals; *equalized feeding*

——— Sprouted maize. ·———· Artificially dried grass.
·–·–·–·–· Control.

When the milk intakes of the animals were "equalized" within groups of three the differences between the weight gains of the groups were, as might be expected, smaller than when the milks were fed *ad libitum*. Thus the greatest difference, that between the control and the dried grass groups, was only 4·2 g. Table XIX shows that neither these small differences nor the slight differences in body length are statistically significant. In this experiment the animals receiving the sprouted maize milk made slightly better gains than the animals which were fed the other two milks.

Regarding the growth performance of the experimental rats, Tables XVII and XVIII show that the gains made by the animals receiving the milks *ad libitum* were definitely greater than the gains made by the "equalized" group. The former groups grew at a rate of about 2·5 g. per day; the latter at about 2·0 g. per day irrespective of the type of milk taken.

As would be expected, the average intake of milk when fed *ad libitum* was greater than when the intake was equalized between litter-mates. This accounts, in part at any rate, for the better growth shown by the animals which were allowed access to unlimited milk. It can be seen from Tables XVII and XVIII that the *ad libitum* animals grew slightly better than the "equalized" ones even when the gain per 100 ml. of milk intake is considered.

Rough bi-weekly records of scouring were kept. They showed that the incidence of scouring was similar for all groups and that after the first week of the experiment it very seldom occurred.

The question of appetite has been recently discussed by White(6). In *ad libitum* feeding experiments there is no difficulty in measuring it, but when the intakes are equalized, special observations and interpretations are necessary. The present experiments are of special interest in this respect as both methods of feeding have been applied to the same milks.

It has already been stated that though in the *ad libitum* feeding experiments some difference in milk intake (appetite) was observed between groups, none of these differences were statistically significant. In Table XX the following statistical tests used for estimating the appetite of the animals on "equalized" milk intakes are given: (I) the number of animals refusing more frequently on one or other type of milk, (II) the total number of refusals, (III) the median quantity of milk refused. The relative merits of these three methods have been discussed by White(6). We find in the present work that method (I), which is known to underestimate the significance of the results, indicates that there is no difference in palatability between the three milks. Method (II), which overestimates the significance, shows that the control milk is more palatable than either of the others and that sprouted maize is more palatable than dried grass milk, but method (III), which is held by White(6) to be a rather more delicate test than either (I) or (II), again fails to indicate any differences in palatability between the experimental milks. It seems that the three types of milk were equally palatable to the rats. This finding agrees with the results of the *ad libitum* feeding tests.

Table XVII. *Various data concerning the growth and the milk intakes of rats receiving the experimental milks.* Ad libitum *feeding*

Litter no.	Rat no.	Weight (g.) Initial	Weight (g.) 2 weeks	Weight (g.) Final	Gain (g.) 10 weeks	Gain (g.) Last 8 weeks	Gain/day (g.) 10 weeks	Gain/day (g.) Last 8 weeks	Milk intake (ml.) 10 weeks	Milk intake (ml.) Last 8 weeks	Gain/100 ml. milk intake (g.) 10 weeks	Gain/100 ml. milk intake (g.) Last 8 weeks	Body length (mm.)
						"Control" milk							
I	6121	45	69	236	191	167	2·73	2·98	5992·0	5275·0	3·19	3·17	220
II	6141	39	58	252	213	194	3·04	3·46	6215·5	5548·0	3·43	3·50	211
III	6146	38	53	227	189	174	2·70	3·11	5505·0	4931·5	3·43	3·53	211
IV *a*	6178	44	74	232	188	158	2·69	2·82	5782·5	5191·5	3·25	3·04	218
IV *b*	6179	44	74	223	179	149	2·56	2·66	5510·0	4897·0	3·25	3·04	211
V	6186	34	50	186	152	136	2·17	2·43	5059·0	4512·5	3·00	3·01	204
VI	6191	43	66	196	153	130	2·19	2·32	5355·5	4737·0	2·86	2·74	202
VII	6226	40	64	193	153	129	2·19	2·30	5013·5	4445·0	3·05	2·90	204
VIII	6233	38	64	196	158	132	2·26	2·36	5420·0	4864·5	2·92	2·71	206
IX	6242	44	70	210	166	140	2·36	2·50	5570·0	4922·5	2·98	2·84	207
Mean for dried grass comparison		40·4	63·7	212·8	172·3	149·1	2·46	2·66	5492·3	4894·4	3·13	3·03	208·2
Mean for sprouted maize comparison		42·6	68·7	212·3	169·7	143·6	2·42	2·56	5520·5	4904·6	3·07	2·92	209·7
						"Artificially dried grass" milk							
I	—	—	—	—	—	—	—	—	—	—	—	—	—
II	6136	39	60	226	187	166	2·67	2·96	5821·0	5172·0	3·21	3·21	212
III	6147	41	58	186	145	128	2·07	2·29	4084·5	3491·0	3·55	3·67	200
IV *a*	6177	45	74	243	198	169	2·83	3·02	5604·0	4994·5	3·53	3·38	214
IV *b*	6176	42	75	244	202	169	2·89	3·02	6148·0	5473·5	3·29	3·09	213
V	6185	32	45	157	125	112	1·79	2·00	4769·5	4261·0	2·62	2·63	194
VI	6192	44	64	190	146	126	2·09	2·25	5298·0	4689·0	2·76	2·69	201
VII	6227	40	62	210	170	148	2·43	2·64	5145·0	4552·5	3·30	3·25	210
VIII	6232	36	60	194	158	134	2·26	2·39	5446·5	4884·5	2·90	2·74	204
IX	6239	44	73	212	168	139	2·40	2·48	5615·5	4948·5	2·99	2·81	206
Mean for control comparison		40·3	63·4	206·9	166·6	143·4	2·38	2·56	5325·8	4718·5	3·13	3·05	206·0
Mean for sprouted maize comparison		41·8	68·0	215·5	173·7	147·5	2·48	2·63	5542·8	4923·7	3·13	2·99	208·0
						"Sprouted maize" milk							
I	6120	44	53	215	171	162	2·44	2·89	5492·0	4869·5	3·11	3·33	217
II	—	—	—	—	—	—	—	—	—	—	—	—	—
III	6145*	41	—	—	—	—	—	—	—	—	—	—	—
IV *a*	6175	44	74	218	174	144	2·49	2·57	5322·0	4751·0	3·27	3·03	211
IV *b*	6180	43	70	204	161	134	2·30	2·39	5311·0	4735·0	3·03	2·83	210
V	6188*	36	—	—	—	—	—	—	—	—	—	—	—
VI	6193	42	64	180	138	116	1·97	2·07	4925·5	4305·5	2·80	2·69	201
VII	6225	39	67	214	175	147	2·50	2·63	5644·0	5036·0	3·10	2·92	214
VIII	6231	36	50	179	143	129	2·04	2·30	4768·0	4318·0	3·00	2·99	203
IX	6243	44	72	219	175	147	2·50	2·63	5540·0	4869·0	3·16	3·02	206
Mean for control comparison		41·7	64·3	204·1	162·4	139·9	2·32	2·50	5286·1	4697·7	3·06	2·97	208·9
Mean for dried grass comparison		41·2	66·2	202·3	161·0	136·2	2·30	2·43	5251·7	4669·1	3·07	2·91	207·5

* These two animals were accidentally lost.

Table XVIII. *Various data concerning the growth and the milk intakes of rats receiving the experimental milks. "Equalized" feeding*

parison																
Mean for dried grass comparison	41·2	66·2	202·3	161·0	136·2	2·30	2·46	5251·7	4669·1	3·07	2·91	207·5				

* These two animals were accidentally lost.

Table XVIII. *Various data concerning the growth and the milk intakes of rats receiving the experimental milks. "Equalized" feeding*

Litter no.	Rat no.	Weight (g.)			Gain (g.)		Gain/day (g.)		Milk intake (ml.)		Gain/100 ml. milk intake (g.)		No. of milk refusals		Total amounts of milk left (ml.)		Body length mm.
		Initial	2 weeks	Final	10 weeks	Last 8 weeks	10 weeks	Last 8 weeks	10 weeks	Last 8 weeks	10 weeks	Last 8 weeks	10 weeks	Last 8 weeks	10 weeks	Last 8 weeks	
								"Control" milk									
I	6196	38	54	168	130	114	1·86	2·04	4870·0	4311·5	2·67	2·64	31	24	202·0	151·0	192
II	6201	46	65	206	160	141	2·29	2·52	5600·0	5015·0	2·86	2·81	20	15	72·0	54 5	211
III	6206	45	59	168	123	109	1·76	1·95	4924·0	4361·0	2·50	2·50	54	45	260·5	203·5	199
IV*a*	6210	47	60	191	144	131	2·06	2·34	5330·0	4746·0	2·70	2·76	6	1	19·5	0·5	210
IV*b*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
V*a*	6216	42	59	147	105	88	1·50	1·57	4330·0	3750·0	2·42	2·35	8	3	41·5	5·5	191
V*b*	6218	40	51	162	122	111	1·74	1·98	4350·0	3840·0	2·80	2·89	2	0	17·0	0	200
VI	6235	35	36	158	123	122	1·76	2·18	4030·0	3610·0	3·05	3·38	3	0	29·5	0	196
Mean for dried grass comparison		43·0	58·0	173·7	130·7	115·7	1·87	2·07	4900·7	4337·3	2·66	2·66	121*	88*	93·1	69·2	200·5
Mean for sprouted maize comparison		41·3	54·2	172·0	130·7	117·8	1·87	2·10	4751·7	4212·1	2·75	2·81	70*	43*	63·6	35·2	200·0
								"Artificially dried grass" milk									
I	6195	42	56	182	140	126	2·00	2·25	4870·0	4310·0	2·87	2·92	21	16	82·5	58·5	200
II	6203	50	70	209	159	139	2·27	2·48	5600·0	5015·0	2·84	2·77	8	5	25·0	11·5	208
III	6205	48	56	162	114	106	1·63	1·89	4925·0	4355·0	2·31	2·43	2	1	16·0	1·5	199
IV*a*	6207	46	59	198	152	139	2·17	2·48	5330·0	4750·0	2·85	2·93	51	44	216·0	190·0	211
IV*b*	6209	39	53	195	156	142	2·23	2·54	5234·0	4692·0	2·98	3·03	39	31	212·5	163·0	204
V*a*	6214	44	54	123	79	69	1·13	1·23	4330·0	3750·0	1·82	1·84	27	23	92·5	71·0	179
V*b*	6215	37	48	152	115	104	1·64	1·86	4350·0	3843·0	2·64	2·71	54	44	271·0	211·0	194
VI	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Mean for control comparison		44·5	57·2	171·0	126·5	113·8	1·81	2·03	4900·8	4337·2	2·56	2·60	163*	133*	117·2	90·6	198·5
Mean for sprouted maize comparison		43·0	56·7	176·5	133·5	119·8	1·91	2·14	4952·3	4393·3	2·67	2·70	200*	163*	149·9	117·5	199·2
								"Sprouted maize" milk									
I	6194	40	54	192	152	138	2·17	2·46	4870·0	4310·0	3·12	3·20	21	19	79·5	73·0	200
II	6202	48	70	200	152	130	2·17	2·32	5599·5	5014·5	2·71	2·59	39	33	184·5	149·0	208
III	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
IV*a*	6208	45	61	196	151	135	2·16	2·41	5330·0	4747·0	2·83	2·84	3	0	19·0	0	211
IV*b*	6211	42	54	199	157	145	2·24	2·59	5232·0	4682·0	3·00	3·10	22	16	126·5	96·5	208
V*a*	6219	42	54	144	102	90	1·46	1·61	4325·0	3748·0	2·36	2·40	43	36	210·5	177·5	190
V*b*	6217	40	52	156	116	104	1·66	1·86	4350·0	3840·0	2·67	2·71	1	0	10·5	0	194
VI	6234	34	35	158	124	123	1·77	2·20	4026·0	3623·0	3·08	3·39	50	40	413·0	320·0	192
Mean for control comparison		41·5	54·3	174·3	132·8	120·0	1·90	2·14	4750·1	4213·8	2·80	2·86	157*	128*	152·8	119·9	199·2
Mean for dried grass comparison		42·8	57·5	181·2	138·3	123·7	1·98	2·21	4951·1	4390·3	2·78	2·81	129*	104*	105·1	82·7	201·8

* These figures = total number of milk refusals.

It has been stated by Kohler *et al.*(18) that in our work on summer and winter milk (Henry & Kon(22)), we used a method "limiting the intake of the rats on summer milk to the level consumed by those on winter milk". This statement is incorrect. We never used such a method. All that is said in the paper is that the consumption of milk was determined in each group by the rat with the smallest appetite. This was not necessarily a winter milk rat, as can be seen from the full report of this work(16).

Table XIX. *Statistical treatment of the differences in gains in weight and in milk intakes of the animals receiving the experimental milks* ad libitum *and in gains in weight of the animals in the "equalized feeding" experiment*

	Ad libitum feeding		"Equalized" intake
	Gain in wt. (g.)	Milk intake (ml.)	Gain in wt. (g.)
Mean: Control (10 weeks)	172·3	5492·3	130·7
Dried grass (10 weeks)	166·6	5325·8	126·5
Difference: control – dried grass	+5·7	+166·5	+4·2
Standard error of mean	±7·46	±185·2	±5·38
*P**	1 : 2 Not significant	1 : 3 Not significant	1 : 2 Not significant
Mean: Dried grass (8 weeks)	147·5	4923·7	119·8
Sprouted maize (8 weeks)	136·2	4669·1	123·7
Difference: dried grass – sprouted maize	+11·3	+254·6	−3·9
Standard error of mean	± 6·51	±175·5	±4·48
*P**	1 : 7 Not significant	1 : 5 Not significant	1 : 2 Not significant
Mean: Sprouted maize (8 weeks)	139·9	4697·7	120·0
Control (8 weeks)	143·6	4904·6	117·8
Difference: sprouted maize – control	−3·7	−206·9	+2·2
Standard error of mean	±4·70	±148·2	±4·96
*P**	1 : 2 Not significant	1 : 5 Not significant	1 : 1 Not significant

* See note to Table XIII.

(*b*) *The second experiment—feeding of milk containing dissolved sugar.* The results of this experiment are given in Tables XXI–XXIV. Table XXI gives the weights (initial, 2 weeks and final), the gains in weight, daily gains in weight, the intakes of milk and sugar solution, the gains for the last 8 weeks per 100 ml. of milk and sugar intake and the body lengths (when killed) of the rats receiving *ad libitum* the experimental milks plus dissolved sugar. Table XXII gives similar figures for the rats for which the intake of milk and sugar was "equalized" within groups of three litter-mates. In addition, it gives the number of days on which each rat left some milk and the total amount of

Table XX. *Statistical treatment of milk refusals of the rats for which the intake of the experimental milks was equalized within groups: (I) the number of animals refusing more frequently on specified milk, (II) the total number of refusals on specified milk, (III) the median quantities (ml.) of milk refused*

	No. of animals refusing more frequently on specified milk	Total number of refusals on specified milk		Median quantity (ml.) of milk refused	
				Control	Dried grass
Control (10 weeks)	3	121	Measurements which define median	41·5, 72·0	82·5, 92·5
Dried grass (10 weeks)	3	163	Median quantity of milk refused (6 rats)	57	88
Difference from mean	0	21	Estimated probable error of median $\left(\sqrt{\frac{\pi}{2n}} \times \text{semi-inter-quartile range}\right)$	±46·69	48·85
Standard error	±1·23	±8·33	Difference: control – dried grass	– 31	
*P**	—	1 : 67	Standard error of mean	±100·20	
	Not significant	Significant	*P**	1 : 1 Not significant	

				Dried grass	Sprouted maize
Dried grass (8 weeks)	3	163	Measurements which define median	71·0, 163·0	73·0, 96·5
Sprouted maize (8 weeks)	3	104	Median quantity of milk refused (6 rats)	117	85
Difference from mean	0	29·5	Estimated probable error of median $\left(\sqrt{\frac{\pi}{2n}} \times \text{semi-inter-quartile range}\right)$	±33·63	±38·13
Standard error	±1·23	±7·97	Difference: dried grass – sprouted maize	+32	
*P**	—	1 : 10,000	Standard error of mean	±75·38	
	Not significant	Significant	*P**	1 : 1 Not significant	

				Sprouted maize	Control
Sprouted maize (8 weeks)	3·5	128	Measurements which define median	73·0, 149·0	0·5, 5·5
Control (8 weeks)	2·5	43	Median quantity of milk refused (6 rats)	111	3
Difference from mean	0·5	42·5	Estimated probable error of median $\left(\sqrt{\frac{\pi}{2n}} \times \text{semi-inter-quartile range}\right)$	±13·94	±45·41
Standard error	±1·21	±5·67	Difference: sprouted maize – control	+108	
*P**	1 : 1	1 : 100,000,000	Standard error of mean	± 70·43	
	Not significant	Significant	*P**	1 : 6 Not significant	

* See note to Table XIII.

milk left by each rat during the experiment. Table XXIII gives a statistical treatment of some of the differences shown in Tables XXI and XXII. Table

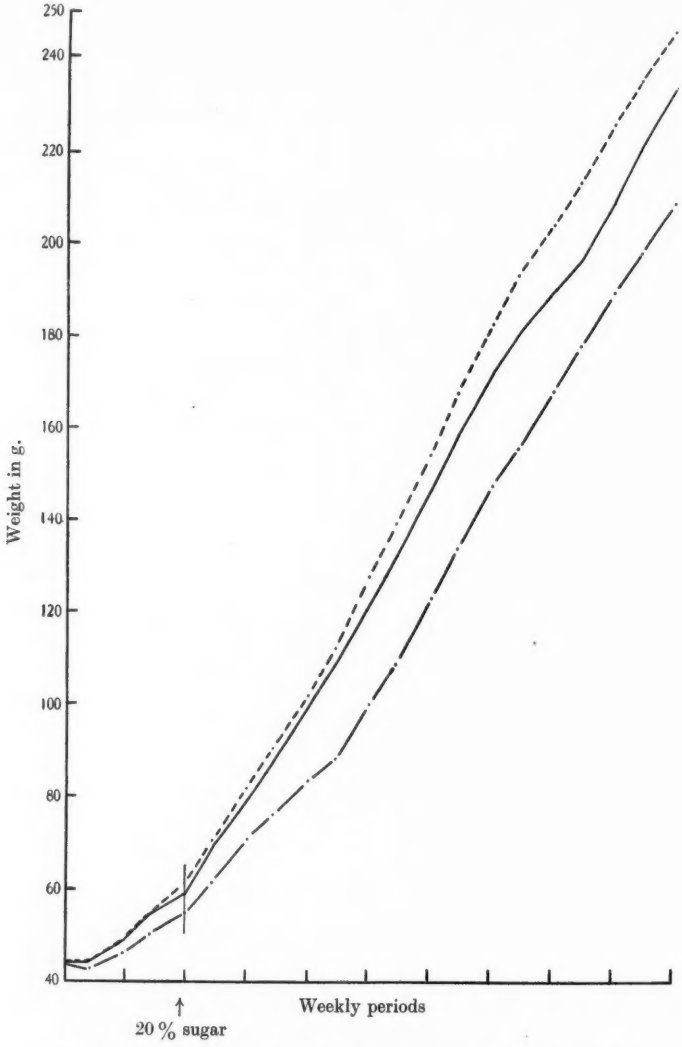

Fig. 7. Average growth curves of animals receiving experimental milks supplemented with minerals and sugar, *ad libitum*

——— Sprouted maize. ·———· Artificially dried grass. ·--------· Control.

XXIV gives statistical tests for estimating the appetite of the "equalized intake" animals. Figs. 7 and 8 give the mean growth curves for the animals receiving the experimental milks.

It can be seen from Table XXI that when the experimental milks containing dissolved sugar were fed to rats, the group receiving the control milk made the best, while the dried grass group made the poorest, gains. Reference to

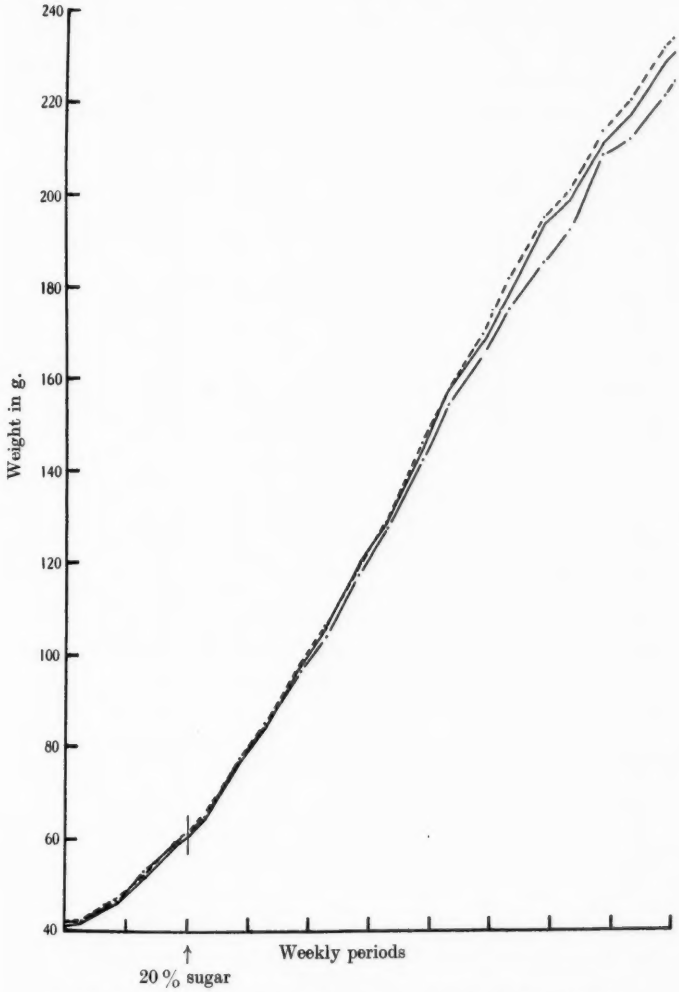

Fig. 8. Average growth curves of animals receiving experimental milks supplemented with minerals and sugar; *equalized feeding*.

——— Sprouted maize. ·———· Artificially dried grass. ·--------· Control.

Table XXIII shows that the superior gains made by the control over the dried grass animals are highly significant statistically, but that the better growth of the sprouted maize as compared with the dried grass group is not quite significant, the probability of 1 : 16 that the difference is due to chance

falling just short of the figure of 1 : 20 which it is customary to accept as significant. The difference between the control and sprouted maize groups is not significant. It will also be seen from Table XXI that the milk and sugar intake of the control group was greatest and that of the dried grass group least, while Table XXIII shows that the consumption of milk and sugar of the

Table XXI. *Various data concerning the growth and the milk intakes of rats on experimental milks containing* 20 *g. of cane sugar*/100 *ml.* Ad libitum *feeding*

Litter no.	Rat no.	Weight (g.) Initial	2 weeks	Final (10 weeks)	Gain (g.)	Gain/day (g.)	Intake of milk + sugar solution (ml.)	Gain/100 ml. milk + sugar intake (g.)	Body Length (mm.)
				"Control" milk					
I*a*	6035	51	72	268	196	3·50	3210·5	6·10	219
I*b*	6036	48	64	203	139	2·48	2438·5	5·70	205
II	6046	36	48	276	228	4·07	3307·0	6·89	224
III	6052	44	65	244	179	3·20	3135·5	5·71	219
IV	6063	43	55	208	153	2·73	2442·0	6·27	211
V	6071	36	54	212	158	2·82	2499·5	6·32	207
VI	6075	48	70	274	204	3·64	3108·5	6·56	219
VII	6238	46	64	281	217	3·88	3552·5	6·11	215
Mean		44·0	61·5	245·7	184·2	3·29	2961·7	6·21	214·9
				"Artificially dried grass" milk					
I*a*	6037	50	60	248	188	3·36	3083·0	6·10	217
I*b*	6038	47	56	226	170	3·04	2660·0	6·39	212
II	6047	35	42	232	190	3·39	2916·0	6·52	215
III	6051	44	54	173	119	2·13	2099·0	5·67	200
IV	6062	42	51	170	119	2·13	1995·0	5·96	198
V	6070	36	52	176	124	2·21	2172·0	5·71	198
VI	6077	47	60	204	144	2·57	2349·5	6·13	207
VII	6240	47	60	244	184	3·29	3000·0	6·13	216
Mean		43·5	54·4	209·1	154·7	2·76	2534·3	6·08	207·9
				"Sprouted maize" milk					
I*a*	6033	50	71	292	221	3·95	3412·0	6·48	225
I*b*	6034	46	66	256	190	3·39	3099·0	6·13	214
II	6044	36	42	220	178	3·18	2671·5	6·66	217
III	6054	44	67	235	168	3·00	2857·0	5·88	214
IV	6061	42	54	201	147	2·63	2286·0	6·43	209
V	6069	36	43	169	126	2·25	1973·0	6·39	191
VI	6078	48	71	266	195	3·48	3363·0	5·80	224
VII	6241	48	61	232	171	3·05	2785·0	6·14	205
Mean		43·7	59·4	233·9	174·5	3·12	2805·8	6·24	212·4

N.B. The experiment proper covered the last 8 weeks.

control group is significantly greater than that of the dried grass group, but that the differences in intake between the control and sprouted maize and between the dried grass and sprouted maize groups are not significant. In this experiment it appears that the addition of sugar to the milks rendered the dried grass milk definitely less palatable to the experimental animals than the control milk. It is not surprising to find, therefore, that when the average gains of the rats are calculated per 100 ml. of milk plus sugar intake, the differences between the three groups become so small as to be statistically

Table XXII. *Various data concerning the growth and the milk intakes of rats on experimental milks containing* 20 *g. of cane sugar*/100 *ml. "Equalized" feeding*

Table XXII. *Various data concerning the growth and the milk intakes of rats on experimental milks containing 20 g. of cane sugar/100 ml. "Equalized" feeding*

Litter no.	Rat no.	Weight (g.) Initial	2 weeks	Final (10 weeks)	Gain (g.)	Gain/day (g.)	Intake of milk + sugar solution (ml.)	Gain/100 ml. milk + sugar intake (g.)	No. of milk Refusals	Total amounts of milk + sugar solution left (ml.)	Body length (mm.)
					"Control" milk						
I	6091	40	58	247	189	3·38	2925·0	6·46	26	142·5	213
II	6100	44	62	206	144	2·57	2640·0	5·45	18	91·5	204
III	6109	46	69	263	194	3·46	3171·0	6·12	31	135·0	220
IV	6116	40	54	212	158	2·82	2535·0	6·23	0	0	210
V*a*	6128	44	63	223	160	2·86	2730·0	5·86	37	157·0	210
V*b*	6129	42	64	225	161	2·88	2734·5	5·89	18	53·0	213
VI*a*	6134*	42	53	180	127	2·27	2324·0	5·46	—	666·0	203
VI*b*	6137	38	56	263	207	3·70	3085·0	6·71	11	46·5	218
Mean for dried grass comparison		41·7	60·5	236·0	175·5	3·14	2848·4	6·14	104†	78·1	213·0
Mean for sprouted maize comparison		42·0	60·9	234·1	173·3	3·10	2831·5	6·10	141†	89·3	212·6
					"Artificially dried grass" milk						
I	6093	40	58	213	155	2·77	2935·0	5·28	6	22·5	210
II	6101	44	63	214	151	2·70	2640·0	5·72	29	151·5	208
III	6108	50	73	269	196	3·50	3175·0	6·17	2	2·5	220
IV	6115	40	54	198	144	2·57	2537·0	5·68	44	377·0	209
V*a*	6133*	43	59	130	71	1·27	1583·0	4·49	—	823·0	181
V*b*	6130	42	63	240	177	3·16	2732·0	6·48	35	269·0	211
VI*a*	6138	40	58	199	141	2·52	2418·0	5·83	16	147·5	208
VI*b*	6140	38	57	236	179	3·20	3079·0	5·81	27	187·0	216
Mean for control comparison		42·3	61·3	228·3	167·0	2·98	2849·7	5·86	143†	168·2	212·3
Mean for sprouted maize comparison		42·0	60·9	224·1	163·3	2·92	2788·0	5·85	159†	165·2	211·7
					"Sprouted maize" milk						
I	6092	40	59	246	187	3·34	2929·5	6·38	20	90·5	214
II	6102	42	62	217	155	2·77	2640·0	5·87	22	129·5	210
III	6111	46	67	264	197	3·52	3173·5	6·21	21	67·0	221
IV	6114	41	49	217	168	3·00	2535·0	6·63	3	20·0	210
V*a*	6131	44	66	226	160	2·86	2730·0	5·86	9	42·5	211
V*b*	6132	41	64	224	160	2·86	2726·0	5·87	19	106·0	215
VI*a*	6135	40	58	208	150	2·68	2440·0	6·15	1	4·0	210
VI*b*	6139	38	58	242	184	3·29	3085·0	5·96	20	92·5	220
Mean for control comparison		41·7	60·7	233·7	173·0	3·09	2831·3	6·11	114†	78·3	214·4
Mean for dried grass comparison		41·1	59·6	231·1	171·6	3·07	2789·9	6·15	106†	72·8	214·3

* These two animals consistently left milk and were finally excluded from their groups; figures for these animals (and their litter-mates where necessary), therefore, are omitted from the averages. Rat 6133 was suffering from respiratory trouble.

† These figures = total number of milk refusals.

N.B. The experiment proper covered the last 8 weeks.

not significant (Table XXIII). The mean body lengths of the three groups of rats increase in the order dried grass, sprouted maize, control. Table XXIII shows that the difference between the control and the dried grass group is just statistically significant, but that the differences in the other two comparisons are not significant. This superiority of the control over the dried grass group can obviously, like the gain in weight, be attributed to the greater milk intake by the control group of rats.

Table XXIII. *Statistical treatment of the differences in gains in weight, in milk plus sugar intakes, in gains/100 ml. of milk plus sugar intake and in body lengths of the animals receiving the experimental milks plus dissolved sugar* ad libitum *and in gains in weight of the animals in the "equalized feeding" milk plus sugar experiment*

	Ad libitum feeding				"Equalized" intake
	Gain in wt. (g.)	Milk + sugar intake (ml.)	Gain/100 ml. milk + sugar intake (g.)	Body length (mm.)	Gain in wt. (g.)
Mean: Control	184·2	2961·7	6·21	214·9	175·5
Dried grass	154·7	2534·3	6·08	207·9	167·0
Difference: control – dried grass	+29·5	+427·4	+0·13	+7·0	+8·5
Standard error of mean	±10·44	±134·9	±0·142	±2·98	±8·19
*P**	1 : 39 Significant	1 : 63 Significant	1 : 3 Not significant	1 : 20 Significant	1 : 3 Not significant
Mean: Dried grass	154·7	2534·3	6·08	207·9	163·3
Sprouted maize	174·5	2805·8	6·24	212·4	171·6
Difference: dried grass – sprouted maize	−19·8	−271·5	−0·16	−4·5	−8·3
Standard error of mean	± 8·94	±166·0	±0·122	±3·47	±6·02
*P**	1 : 16 Not significant	1 : 7 Not significant	1 : 4 Not significant	1 : 4 Not significant	1 : 5 Not significant
Mean: Sprouted maize	174·5	2805·8	6·24	212·4	173·0
Control	184·2	2961·7	6·21	214·9	173·3
Difference: sprouted maize – control	− 9·7	−155·9	+0·03	+2·5	−0·3
Standard error of mean	±12·21	±175·2	±0·135	±3·06	±4·26
*P**	1 : 2 Not significant	1 : 2 Not significant	1 : 1 Not significant	1 : 2 Not significant	1 : 1 Not significant

* See note to Table XIII.

When the results of the "equalized" experiment are considered, it is found that the differences in gain in weight and in body length between the three groups of animals are very small and are not significant (Table XXIII). The small differences between the gains in weight of the three groups are in the same direction as those observed when the milks plus sugar were fed *ad libitum*.

The average daily gain of about 3 g. was independent of the method of feeding, a finding which is contrary to that obtained when milk was fed alone. This similarity in the growth rate can probably be attributed to the fact that

Table XXIV. *Statistical treatment of milk plus sugar refusals of the animals for which the intakes of the experimental milks were equalized within groups: (I) the number of animals refusing more frequently on specified milk, (II) the total number of refusals on specified milk, (III) the median quantities (ml.) of milk plus sugar solution refused*

	No. of animals refusing more frequently on specified milk	Total number of refusals on specified milk		Median quantity (ml.) of milk refused	
				Control	Dried grass
Control	2	104	Measurements which define median	91·5, 53·0	187·0, 151·0
Dried grass	4	143	Median quantity of milk refused (6 rats)	72	169
Difference from mean	1	19·5	Estimated probable error of median $\left(\sqrt{\frac{2\pi}{n}} \times \text{semi-inter-quartile range}\right)$	±22·64	±63·06
Standard error	±1·15	±7·76	Difference: control – dried grass	−97	
*P**	1 : 4	1 : 100	Standard error of mean	±99·34	
	Not significant	Significant	*P**	1 : 3 Not significant	
				Dried grass	Sprouted maize
Dried grass	5	159	Measurements which define median	—	—
Sprouted maize	2	106	Median quantity of milk refused (7 rats)	151·0	90·5
Difference from mean	1·5	26·5	Estimated probable error of median $\left(\sqrt{\frac{2\pi}{n}} \times \text{semi-inter-quartile range}\right)$	±33·88	±13·20
Standard error	±1·20	±7·98	Difference: dried grass – sprouted maize	+60·5	
*P**	1 : 5	1 : 1000	Standard error of mean	±53·91	
	Not significant	Significant	*P**	1 : 3 Not significant	
				Sprouted maize	Control
Sprouted maize	4	114	Measurements which define median	—	—
Control	3	141	Median quantity of milk refused (7 rats)	90·5	91·5
Difference from mean	0·5	13·5	Estimated probable error of median $\left(\sqrt{\frac{2\pi}{n}} \times \text{semi-inter-quartile range}\right)$	+21·09	±10·54
Standard error	±1·31	±7·94	Difference: sprouted maize – control	− 1·0	
*P**	1 : 2	1 : 11	Standard error of mean	±34·94	
	Not significant	Not significant	*P**	1 : 1 Not significant	

* See note to Table XIII.

the actual gains in weight and the milk intakes (except when the dried grass milk was fed *ad libitum*) were practically unaffected by the method of feeding.

The better rate of growth shown by the two sets of animals receiving sugar with their milk over those receiving milk alone is connected with a higher calorie intake of the former. The addition of 20 g. sugar per 100 ml. of milk doubled the energy value of the milk. The average daily intake of milk for the last 8 weeks by the rats receiving milk alone was 88 and 79 ml. for the *ad libitum* and "equalized" feeding respectively, corresponding to daily intakes of 59 and 53 Cal. respectively[1]. When the sugar was added to the milk, the average daily intake of the milk solution was about 50 ml. (44 ml. milk), yielding 70 Cal.

It has already been mentioned that when sugar was added to the experimental milks, fed *ad libitum*, the dried grass milk was less palatable to the animals than the other two (Table XXIII). Table XXIV gives the statistical methods of measuring the appetite of the animals receiving the three types of milk when the milk plus sugar intake was "equalized" within the groups of three. Method I, which uses the number of refusals on a greater number of days, indicates no differences in the palatability of the three milks; Method II, using the total number of refusals, indicates that the control and sprouted maize milks are more palatable than the dried grass milk, but that the palatabilities of the control and sprouted maize milks are statistically indistinguishable. This method is known, however, to overestimate the significance of any differences. Method III, which employs the median quantity of milk refused, fails to indicate any differences in the palatabilities of the three milks. "Equalized" feeding experiments, therefore, fail to demonstrate the lowered appetite shown by the dried grass group of rats when the milk plus sugar was fed *ad libitum*.

Discussion

The *ad libitum* feeding experiments in which an exclusive diet of mineralized milk was used disclosed no differences between the three experimental milks. Several explanations may be advanced to account for this finding.

If, in the first instance, the views of the Wisconsin workers regarding the fluctuation in the growth-promoting properties of milk are accepted, it might be that none of the additions to the winter ration of the cows exerted any beneficial effect. If so, the concentration of the specific factor concerned with appetite would be at its lowest in all three milks and the growth of rats would be reduced to the same common level. This assumption would be quite in keeping with the views of Kohler *et al.* (18), who have shown that the "grass juice" factor content of artificially dried forages varies considerably, and that sprouted seedlings such as oats seedlings grown in a greenhouse may be altogether devoid of the factor. Another argument in favour of this explana-

[1] The caloric value of the milk was calculated by the method of Overman & Sanmann (23) from the data given in Table XVI.

tion will be found in the growth rate of our animals which was well within the winter limits cited by these authors. As our experiments lasted 10 weeks against the usual 6 weeks of the American investigators, we have also calculated the average daily gains for the first six weeks of the experiment. They were 2·5 g. for each of the milks.

Against these arguments it should be pointed out, however, that, though Kohler *et al.* have assumed that the factor which is responsible for the acceleration of the growth of rats when lawn clippings or grass juice are added to mineralized winter milk is identical with the factor responsible for the difference observed by them between winter and summer milk, no experimental proof of this assumption has been given.

A more serious objection is provided by the behaviour of the rats which, in *ad libitum* feeding experiments, received cane sugar in addition to the mineralized milks. The rats took an average of some 50 ml. daily of the milk plus sugar solution, containing about 44 ml. of milk, as against 88 ml. of milk taken over the same period of time by the rats which received, *ad libitum*, only mineralized milk. Yet all three groups of rats receiving sugar made better gains than the corresponding groups on milk alone. This is incompatible with the assumption that the growth of the rats on the exclusive diet of milk was restricted by a limiting factor unless one assumes, in addition, either that the factor is present in pure cane sugar or that sugar markedly increases the utilization of milk. The first supposition is highly improbable and the second is not in agreement with the facts. It will be seen from Tables XXI and XVII that the gain per 100 ml. of intake for the milk plus sugar rats was almost exactly double that of the rats taking milk alone. As the caloric values of the two liquids were in the same ratio, they were equally well utilized.

It might be mentioned here that the rats of Kohler *et al.*(18) utilized milk much better than the rats used in the present experiments. Thus the second control group of males (their Table III) made a gain of almost 7 g. per 100 ml. of milk intake, i.e. more than twice the value observed by us (Table XVII). It can be calculated that the milk used by Kohler *et al.* contained 14·5 % total solids against 12·3 % in our milks, but this is not enough to explain the above difference. The periods in the two sets of experiments are also not comparable, but even if our results were calculated for the period corresponding to that for which Kohler *et al.* give figures, we should obtain nothing like the efficiency of utilization observed in their rats.

Another tentative explanation would be to assume that all three diets supplied more than enough of the appetite-controlling factor and that the rats were growing at maximal rate. Here again, the acceleration of growth on addition of sugar would be inexplicable. To assume that despite the special treatment, the rats were not "run out" and carried stores of the factor, would be still another possibility. This in itself would not explain why rats grew at the same rate on the three experimental milks, nor why the addition of sugar improved the growth.

It will be seen, therefore, that our results do not support the view that a new essential substance is the first limiting factor of mineralized milks. As before (Henry & Kon (19), Henry *et al.* (16) and Henry *et al.* (20)), the results are most easily explained on the assumption that, in exclusive milk feeding, the margin of safety in the supply of various factors required by the rat is too great for differences between various milks to become manifest.

It is possible, of course, that our failure to confirm the findings of the Wisconsin workers is due to differences between the English and American methods of feeding and management of cows. The fact remains, however, that we have been unable to use the method of exclusive milk feeding as a means of detecting nutritive differences between milks. It may be noted that since the present experiments were completed, we have carried out similar studies on dried and evaporated milks, with identical results.

Even in cases where the method proves successful it would not be correct to describe it as measuring the nutritive value (or, as we have called it in earlier papers, the total nutritive value) of milk. Dealing with a limiting factor it would yield information concerning the concentration in milk of this factor alone without reference to the nutritive properties of other milk constituents.

SUMMARY

1. An experiment is described in which comparisons were made between the effect of (*a*) mangels (control feeding), (*b*) artificially dried grass, (*c*) sprouted maize, and (*d*) kale used as dietary supplements on the production and nutritive properties of the milk of dairy cows.

2. No significant differences were found between any of the foods in their effect on yield of milk, or its content of fat or solids-not-fat.

3. The feeding of kale and of artificially dried grass markedly increased the colour and vitamin A content of the milk, but sprouted maize had no more effect in this respect than mangels.

4. As compared with the feeding of mangels, sprouted maize and artificially dried grass had no measurable effect on the vitamin B complex level of the milk.

5. The vitamin B_2 (complex) level was more variable and was lowest in the milk from the sprouted maize cows. It is not known, however, whether the difference was due to the feeding or to differences in the cows themselves.

6. The same statement applies to figures for the vitamin C level which was highest in the sprouted maize milk.

7. The growth-promoting properties of the milks produced on mangels, artificially dried grass and sprouted maize were compared. The milks were fed, supplemented either with minerals alone, or with minerals plus sugar as a source of energy. In one series of experiments the milks were given *ad libitum*, in another the intake was "equalized." No differences were found and it is concluded that under English conditions, this technique does not disclose the presence in milk of a new essential factor.

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197. THE COMPOSITION OF THE BLOOD AND MILK OF LACTATING COWS DURING INANITION, WITH A NOTE ON AN UNIDENTIFIED CONSTITUENT PRESENT IN CERTAIN SAMPLES OF ABNORMAL MILK

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Except for a publication by Overman & Wright (1) in 1927 and one by Gowen & Tobey (2) in 1931, the literature of the past four decades contains very little information on the nature of milk secreted by animals during a period of starvation. The first paper records the variations in the yield and composition of the milk of three cows which were starved for 5 or 6 days. The authors' general conclusions were that inanition causes a decrease in both the yield and the lactose content of milk, and that this is accompanied by an increase in the percentage of total solids, fat, protein and ash. In the work of Gowen & Tobey several cows were starved for times varying from 2 to 3½ days. The milk was analysed for a number of constituents, while the sugar and calcium contents of the blood were also estimated. In neither of these two investigations was the general metabolism of the animals studied before and during the fast, nor were changes in the nature of the protein and fat of either the blood or the milk considered. The periods during which food was withheld were, moreover, too short to allow basal metabolism to be reached in ruminants. It was therefore decided to study in detail the secretion of milk by cows during a 12-day fast with the object of determining (*a*) whether any variations occurred in the yield and composition of the milk, and (*b*) whether these variations could be related to corresponding changes either in the blood constituents of the animals or in their metabolism as a whole. It was felt that such information might provide valuable evidence regarding the nature of the milk precursors in the blood and their conversion into milk constituents by the mammary gland. The work therefore included both milk and blood analyses, as well as a study of the general metabolism of the experimental animals. The present communication is concerned with the two former subjects, i.e. the yield and composition of the milk, and the level of certain constituents of the blood.

Experimental

The animals used for the present experiment were three non-pregnant Ayrshire cows in the sixth to seventh month of their lactation period. At the end of a few weeks, during which the animals had been receiving a normal well-balanced diet, food was withheld from them for a period of 12 days,

after which free access to food was again permitted.[1] Water was at all times given freely. Before, during and after the 12-day fast the daily yields of milk were measured and composite daily samples analysed. The substances estimated were total nitrogen (Kjeldahl), lactose (polarimetrically), fat (Gerber), chlorides (Davies(3)), phosphatase (Bodansky(4)) and total solids. For certain samples ash was also determined, while a study of the nitrogen partition was made twice before and several times during starvation and, for two of the cows, twice after realimentation was established. For this purpose a slight modification of the method of Moir (1931) was used, the figures for total, for non-protein, and for non-protein *plus* albumin-globulin nitrogen being first estimated and those for the total protein and casein nitrogen being obtained by difference. In regard to blood analyses the following constituents and methods were chosen: total nitrogen (Kjeldahl), serum albumin and globulin (Howe), amino-acid nitrogen (Folin), non-protein nitrogen (micro-Kjeldahl), uric acid (Folin), calcium (Collip), phosphorus and phosphatase (Bodansky), sugar (a macro form of Hagedorn-Jensen's method using 5 ml. plasma), and chloride (Davies(3)). Plasma was used for the chloride and sugar analyses, and serum for the remainder.

The results for the milk analyses are recorded in Tables I–III, in which, owing to the possible importance of daily changes in yield and composition during the starvation period, it seemed desirable to quote the figures for every day of the fast. The results for the blood analyses are recorded in Tables IV and V.

Discussion

Before proceeding to a detailed discussion of the results it should be explained that cow 1 was the only animal of the three to give an example of simple starvation free, as far as could be ascertained, from any other complication. With cow 2 the general condition of inanition was accompanied on the third day of the fast by some illness akin in its symptoms to milk fever. It will be observed from Table V that a blood calcium figure of 8·5 for this animal in the pre-fast period fell to 5·8 during the brief illness. After inflation of the udder the cow appeared to recover, and in subsequent blood analyses there was a return towards the normal calcium value. The attack appeared to last for only a few hours, but its occurrence must be taken into account in discussing the present results. With cow 3, the fast was carried through until the seventh day when, owing to the animal's unsatisfactory condition, the experiment was discontinued. It may be noted that this animal was in somewhat poor condition at the commencement of the fast, and on subsequent *post mortem* examination was found to be almost completely devoid of depot fat. Up to the sixth or seventh day the fast may, however, probably be looked upon as a case of simple inanition.

[1] The present results were obtained in the course of an experiment undertaken by Dr S. Morris to determine the effect of inanition on the metabolism of lactating and non-lactating ruminants.

Table I. *The yield and composition of milk from cow* 1

Days of fast	Yield l.	Total nitrogen g./100 ml.	Nitrogen partition as % of total nitrogen: Protein	Albumin plus globulin	Casein	Non-protein	Fat %	Lactose %	Chloride %	Solids-not fat %	Solids-not-fat less the sum of protein, lactose and ash %	Phosphatase units per 100 ml.
Before	16·18	0·48	93·3*	15·1	78·2	6·7	3·10	4·28	0·100	8·09	0·0	35
			93·7*	15·0	78·7	6·3						
During 1	11·72	0·49	—	—	—	—	4·08	4·17	0·101	8·23	0·1	54
2	6·97	0·47	89·8	17·5	72·3	10·2	5·54	3·80	0·098	8·07	0·5	58
3	5·79	0·47	—	—	—	—	6·10	4·17	0·101	8·24	0·3	68
4	5·12	0·48	92·5	16·7	75·8	7·5	7·00	4·28	0·103	8·39	0·3	96
5	4·45	0·52	—	—	—	—	7·95	4·12	0·107	8·32	0·1	145
6	3·76	0·54	93·5	19·0	74·5	6·5	8·40	4·06	0·115	8·67	0·4	152
7	3·40	0·53	—	—	—	—	8·90	4·17	0·119	8·55	0·2	146
8	3·24	0·54	93·9	16·0	77·9	6·1	9·13	4·06	0·122	8·63	0·3	129
9	2·80	0·58	—	—	—	—	9·67	3·89	0·127	8·71	0·3	143
10	2·44	0·57	93·0	19·0	74·0	7·0	9·02	4·01	0·128	9·06	0·5	141
11	2·55	0·57	—	—	—	—	8·89	4·12	0·134	8·72	0·2	161
12	2·72	0·56	92·7	18·5	74·2	7·3	8·60	4·21	0·136	8·61	0·0	151
After 1	2·44	0·53	—	—	—	—	7·48	4·14	0·146	8·74	0·4	122
2	2·72	0·57	—	—	—	—	6·90	3·84	0·151	8·35	0·1	133
3	3·25	0·56	92·1	23·5	68·6	7·9	6·00	3·60	0·163	8·30	0·3	—
5	5·92	0·50	92·3	22·5	69·8	7·7	4·30	4·04	0·157	8·21	0·2	—
16	11·00	0·61	—	—	—	—	2·65	4·42	0·116	9·34	0·2	—

* In Tables I–III the nitrogen figures for the pre-fast period were determined for two composite samples of milk taken on different days. The other pre-fasting figures were for a composite daily sample taken either 1 or 2 days before inanition and were in each case typical of the milk for some time before the fast began.

Table II. *The yield and composition of milk from cow* 2

Days of fast	Yield l.	Total nitrogen g./100 ml.	Nitrogen partition as % of total nitrogen: Protein	Albumin plus globulin	Casein	Non-protein	Fat %	Lactose %	Chloride %	Solids-not fat %	Solids-not-fat less the sum of protein, lactose and ash %	Phosphatase units per 100 ml.
Before	15·00	0·49	93·3*	15·1	78·2	6·7	3·10	4·28	0·100	8·09	0·0	35
			93·7*	15·0	78·7	6·3						
During 1	10·00	0·50	—	—	—	—	4·08	4·17	0·101	8·23	0·1	54
2	6·00	0·48	89·8	17·5	72·3	10·2	5·54	3·80	0·098	8·07	0·5	58
3	5·00	0·48	—	—	—	—	6·10	4·17	0·101	8·24	0·3	68
4	4·50	0·49	92·5	16·7	75·8	7·5	7·00	4·28	0·103	8·39	0·3	96
5	4·00	0·53	—	—	—	—	7·95	4·12	0·107	8·32	0·1	145
6	3·50	0·55	93·5	19·0	74·5	6·5	8·40	4·06	0·115	8·67	0·4	152
7	3·00	0·54	—	—	—	—	8·90	4·17	0·119	8·55	0·2	146
8	2·75	0·55	93·9	16·0	77·9	6·1	9·13	4·06	0·122	8·63	0·3	129
9	2·50	0·59	—	—	—	—	9·67	3·89	0·127	8·71	0·3	143
10	2·25	0·58	93·0	19·0	74·0	7·0	9·02	4·01	0·128	9·06	0·5	141
11	2·00	0·58	—	—	—	—	8·89	4·12	0·134	8·72	0·2	161
12	1·75	0·57	92·7	18·5	74·2	7·3	8·60	4·21	0·136	8·61	0·0	151
After 1	1·50	0·54	—	—	—	—	7·48	4·14	0·146	8·74	0·4	122
2	1·25	0·58	—	—	—	—	6·90	3·84	0·151	8·35	0·1	133
3	1·00	0·57	92·1	23·5	68·6	7·9	6·00	3·60	0·163	8·30	0·3	—
5	0·75	0·51	92·3	22·5	69·8	7·7	4·30	4·04	0·157	8·21	0·2	—
16	0·50	0·62	—	—	—	—	2·65	4·42	0·116	9·34	0·2	—

Table II. *The yield and composition of milk from cow* 2

Days of fast	Yield l.	Total nitrogen g./100 ml.	Nitrogen partition as % of total nitrogen: Protein	Albumin plus globulin	Casein	Non-protein	Fat %	Lactose %	Chloride %	Solids-not-fat %	Solids-not-fat less the sum of protein, lactose and ash %	Phosphatase units per 100 ml.
Before	15·00	0·52	93·1*	15·8	77·3	6·9	4·20	4·87	0·095	9·31	0·3	44
			93·9*	15·5	78·4	6·1						
During 1	11·40	0·50	91·5	15·3	76·2	8·5	5·05	4·83	0·100	8·92	0·1	47
2	4·96	0·51	—	—	—	—	6·85	4·56	0·095	8·88	0·2	44
3†	0·15	0·95	88·2	28·3	59·9	11·8	6·20	0·75	0·250	8·32	0·7	44
4	0·16	1·10	—	—	—	—	8·30	0·47	0·229	9·56	1·3	—
5	0·46	1·23	93·4	35·3	58·1	6·6	15·40	0·65	0·211	10·52	1·2	152
6	0·53	1·18	—	—	—	—	14·80	0·62	0·228	10·72	1·8	164
7	0·64	1·19	95·0	31·9	63·1	5·0	15·30	0·69	0·220	10·19	1·1	164
8	0·70	1·05	—	—	—	—	14·10	0·49	0·227	9·80	1·8	188
9	0·86	0·96	94·3	28·4	65·9	5·7	11·70	1·63	0·224	9·45	0·9	150
10	1·00	0·93	—	—	—	—	11·70	2·11	0·210	9·80	1·0	169
11	1·00	0·87	93·7	31·9	61·7	6·2	10·10	2·12	0·208	9·43	1·0	148
12	1·13	0·88	—	—	—	—	9·00	2·31	0·209	9·50	0·8	144
After 1	0·96	0·87	90·1	30·7	59·4	9·9	8·75	2·00	0·211	8·97	0·6	99
2	0·91	0·80	—	—	—	—	6·80	1·27	0·252	7·96	0·8	104
3	1·51	0·76	—	—	—	—	6·20	1·71	0·247	8·14	0·8	99
4	2·37	0·69	93·7	26·6	67·1	6·3	4·80	2·89	0·230	8·67	0·6	70
10	6·12	0·61	94·6	19·2	75·4	5·4	2·90	4·42	0·142	9·20	0·2	65
40	8·62	0·56	—	—	—	—	3·93	5·23	0·096	9·77	0·2	—

* See footnote to Table I. † Animal affected with milk fever.

Table III. *The yield and composition of milk from cow* 3

Days of fast	Yield l.	Total nitrogen g./100 ml.	Nitrogen partition as % of total nitrogen: Protein	Albumin plus globulin	Casein	Non-protein	Fat %	Lactose %	Chloride %	Solids-not-fat %	Solids-not-fat less the sum of protein, lactose and ash %	Phosphatase units per 100 ml.
Before	13·94	0·58	95·1*	11·8	83·3	4·9	3·68	5·08	0·084	9·72	0·2	32
			95·1*	12·8	82·3	4·7						
During 1	9·10	0·59	—	—	—	—	4·40	4·90	0·085	9·58	0·1	49
2	3·96	0·63	92·8	12·9	79·8	7·2	7·40	4·40	0·088	9·60	0·4	51
3	2·30	0·68	—	—	—	—	7·92	4·12	0·110	9·57	0·3	75
4	1·36	0·67	94·2	16·4	77·9	5·8	7·92	3·80	0·152	8·80	−0·1	80
5	0·90	0·70	—	—	—	—	9·90	3·14	0·172	8·86	0·5	144
6	0·62	0·79	94·9	19·6	75·3	5·1	11·00	2·58	0·184	8·85	0·5	178
7	0·16	0·90	—	—	—	—	13·86	0·49	0·179	8·93	1·9	181

* See footnote to Table I.

Table IV. *The nitrogen partition in the blood serum*

Days of fast	Total protein g./100 ml.	Albumin g.	Globulin g.	Non-protein-nitrogen mg.	Urea nitrogen mg.	Amino-acid nitrogen mg.	Residual non-protein nitrogen mg.
			Cow 1				
Before	7·50	4·34	3·16	17·7	8·2	7·1	2·4
	7·60	4·62	2·98	19·2	8·0	6·4	4·8
	7·93	4·78	3·15	19·8	9·0	6·4	4·4
During	8·06	5·12	2·94	22·1	9·0	6·9	6·2
	8·50	5·21	3·29	28·7	10·3	7·4	11·0
	8·35	5·02	3·33	31·9	11·7	7·5	12·7
After	7·21	4·48	2·73	22·4	10·7	6·6	5·1
	7·93	4·78	3·15	17·9	11·0	6·9	—
			Cow 2				
Before	—	4·13	—	15·7	7·9	6·7	1·1
	7·89	4·19	3·70	19·6	8·0	6·4	5·2
During	9·44	5·47	3·97	24·5	8·9	7·1	8·5
	8·22	4·96	3·26	24·1	11·3	6·3	6·5
	8·17	4·86	3·31	32·2	9·7	7·6	14·9
	8·21	4·84	3·37	24·0	13·0	7·2	3·8
After	7·61	4·05	3·56	23·8	9·5	6·7	7·6
	8·06	4·74	3·32	17·8	10·4	6·9	0·5

Table V. *The chloride, sugar, phosphatase and corpuscular volume of the blood*

Days of fast	Corpuscular volume (%)	Phosphatase units/100 ml. serum	Calcium mg./100 ml. serum	Chlorides (mg./100 ml.)		Sugar mg./100 ml. plasma
				Whole blood	Plasma	
			Cow 1			
Before						
2	33	4·5	10·2	305	363	56
1	32	4·7	10·6	306	368	54
Just prior	31	5·0	10·5	279	346	48
During						
5	40	3·0	9·8	275	347	63
10	41	4·1	10·2	267	337	68
11	42	3·2	8·1	267	323	71
After						
23	33	4·8	10·6	296	365	58
28	32	4·2	10·8	307	375	87†
30	35	—	—	287	352	64
			Cow 2			
Before						
8	33	—	8·4	303	359	54
5	34	—	—	298	360	54
1	33	5·0	8·6	306	355	54
During						
2	43*	3·9	5·8*	271	343	208*
6	40	5·7	7·9	283	338	66
8	38	5·5	7·5	282	334	68
12	36	6·0	7·8	272	323	72
After						
38	29	4·7	8·6	305	357	79
40	30	5·4	9·8	301	360	59
44	31	—	—	301	357	68

* Milk fever.

† This blood sample was obtained with some difficulty which may account for the higher value.

Milk yields

It will be seen from Table I that the milk yield in simple starvation (cow 1) fell rapidly in the first few days to less than a third of its initial value. It then diminished more slowly until by the tenth day it was reduced to 2·44 l.—about one-seventh of the original figure. Subsequent variations were slight until the third day of realimentation, after which the yield increased rapidly and ultimately approached very closely to the figure which would have been expected from an uninterrupted normal lactation. Some weeks after the experiment, however, the quality of the milk was still somewhat impoverished, particularly in its fat content. For the other two cows the yield fell much more rapidly. This is probably explained by the onset of milk fever in cow 2 and by the lack of body reserves in cow 3. It is of interest to notice that as soon as cow 2 recovered from the milk fever, the yield steadily increased from the low figure of 0·15 to 1·13 l. on the twelfth day of the fast, although food was rigidly withheld during that period. After realimentation the yield further increased until some 6 weeks later it had reached about 60 % of the original pre-fast value.

The protein content and nitrogen partition

The protein content of the milk of all three cows rose considerably. In simple starvation there was an increase of nearly 20 % by the ninth day, and this high value persisted with slight fluctuations for at least 16 days after realimentation. In cow 2 the onset of milk fever was accompanied by almost a 90 % increase in protein nitrogen, and this rose to well over 100 % by the seventh day although there was a simultaneous rise in the volume of the milk. The protein content then fell slowly, but after 10 days of the post-fast period it was still some 20 % above the original value. With cow 3 the protein nitrogen rose by some 50 % during the 7 days of the experiment. It may be noted that the very large increase in the protein content of the milk of cow 2 after milk fever is similar to that obtained by Mattick & Wright(9) in the milk of a cow which had been caused to ingest large amounts of ammonium chloride. A few days after the salt ingestion, when the cow was recovering from its immediate and somewhat severe after-effects, the protein of the milk rose as high as 7·5 % while the fat increased to 17 %, a figure which was also paralleled by cow 2 in the present work.

When the figures for the nitrogen partition are considered it is found that the proportion of nitrogen present as protein did not vary markedly, though there was a decided temporary fall in the initial stages of the fast. Thus the initial values of 93·5, 93·5 and 95·1 % for cows 1, 2 and 3 fell to 89·8, 88·2 and 92·8 % in the first 2 or 3 days of the fast, after which they returned quickly to the original values. With regard to the proportion of nitrogen present as albumin *plus* globulin and as casein, the former increased slightly during the fast at the expense of the latter. With simple starvation this change took place

slowly and did not reach its maximum by the end of the fast. Thus an initial value of 78·5 % for the proportion of casein nitrogen fell to 74·2 % by the twelfth day, while 4 or 5 days after the fast it was still lower, i.e. 69 %, the corresponding values for the albumin *plus* globulin nitrogen being 15·0, 18·5 and 23·0 %. Where milk fever intervened the albumin *plus* globulin nitrogen reached a maximum by the fifth day, 15·7 rising to 35·3 %, while by the tenth day of the post-fast period it had decreased to 19·2 %. With cow 3 it rose from 12 to 20 % during the 7 days of the fast.

It was hoped at the beginning of the work that it might be possible to trace the cause of any marked changes in these constituents to changes in the nitrogenous constituents of the blood. But it was found most difficult to draw any reliable conclusions on this point. Reference to Table IV shows that during starvation there was an increase in the total protein, albumin, non-protein nitrogen and residual nitrogen fractions of the blood serum, while the globulin fraction remained almost unchanged. In particular the pre-fasting figures for albumin for cows 1 and 2 were approximately 4·6 and 4·2 mg./100 ml. and these rose to over 5 mg. as a result of inanition. It might perhaps be thought at first sight that the cause of the rise in the albumin *plus* globulin fraction of the milk could be traced to this increase in the albumin content of the blood. It must, however, be borne in mind that when the normal processes of the mammary gland are disturbed there is usually a tendency for the albumin-globulin fraction of the milk to increase at the expense of the casein, and that this may simply be due to a disturbance, within the gland itself, of the processes normally leading to the synthesis of casein, with a consequent decrease in the proportion of this particular protein and a corresponding rise in that of the others. The question whether, in the present experiments, changes in the blood albumin level specifically affected the albumin content of the milk must therefore be considered an open one.

The fat content of the milk

The percentage of fat in the milk of all three animals rose considerably as the volume fell, figures being obtained as high as 9·7 % in simple starvation, and as high as 15·4 % when milk fever was a complicating factor. But when the total daily yield of fat is considered as distinct from the percentage content it is found that during starvation the output fell daily throughout the fast and continued to do so until realimentation had been 2 days in progress. Thus with cow 1 the initial daily yield of 568 g. fell to 234 g. by the twelfth day of the fast. Similar results were obtained with the other two cows. A detailed study of the nature of the fat and its relationship to the amount and nature of the blood lipoids before, during and after inanition is at present nearing completion and will be published in the near future. It may be noted in passing that a very marked and progressive change was found in the nature of the milk fat for all three cows as the fast proceeded.

The lactose and chloride contents

It is a well-established fact that lactose and chlorides account for some 75 % of the osmotic pressure of milk, and it is generally believed that a high chloride figure is usually paralleled by a correspondingly low value for lactose, so that the isotonicity of the serum is maintained. Formulae have in fact been deduced by which it is claimed that the approximate lactose content of milk can be calculated from the chloride content. That this is true for normal milks has been confirmed from time to time by various authors (cf. Davies(3)), but the present results show that the statement must be accepted with reserve in dealing with even a slightly abnormal type of milk. For example, from Table I it will be seen that between the first and second days of the fast both the chloride and lactose contents tended to decrease, while if the figures for the second day be compared with those for the ninth, the lactose content is seen to vary to the extent of only 2–3 %, whereas the chloride alters by about 30 %. Reference to the tables will confirm the fact that several other examples of a similar nature could be cited.

A less definite relationship has been postulated by Koestler(5), who has suggested that for normal milk the ratio $\frac{\text{chloride content} \times 100}{\text{lactose content}}$ should not exceed 3·5 and should usually lie between 1 and 3, but that when the udder is disturbed this ratio may vary between 0·5 and 15. It is interesting to observe that when the Koestler number is calculated for cow 1 during simple starvation, the value never exceeded 3·3 and was never less than the initial figure of 2·3. In fact, milk which would be classed as abnormal from this point of view was only obtained from cow 1 during the first few days of realimentation, when a maximum figure of 4·5 was obtained. With cow 2, where milk fever intervened, the Koestler number rose from about 2 before the fast to the very high figure of 33 on the third day, and by the eighth day it had reached its maximum at 46, returning almost to normal by the tenth day of realimentation. Again with cow 3, where inanition was complicated by serious loss of condition, the value rose from 1·7 to 7·1 on the sixth day of the fast and was as high as 37 on the seventh day. That the abnormal values are due to the effects of milk fever or other abnormal conditions rather than to true starvation is suggested first by the fact that it scarcely occurred at all with cow 1, and secondly by the fact that with cow 2 a return towards the normal value began 4 days before the fast ended. It would appear, therefore, that, as far as the Koestler number is concerned, the milk secreted during simple starvation is normal. It will be observed that where the abnormality occurs it is accompanied by very low lactose contents, a fact to which further reference is made in the next section.

Solids-not-fat and the presence of an unidentified constituent in abnormal milk

It will be unnecessary to discuss the solids-not-fat in detail, since full reference has already been made to the main constituents separately. It will suffice to draw attention to the fact that during the first 3 days of simple starvation (Table I) the solids-not-fat varied only slightly from the initial value of 8·09 %, after which they increased gradually to 9·06 %, a slight temporary fall again occurring on the last 2 days of the fast and on realimentation.

A peculiar and possibly most significant observation was made in connexion with the solids-not-fat of the milk secreted during the fast by cows 2 and 3. This was the fact that an unidentified constituent appeared to be present—a constituent which was not estimated as protein, lactose or ash and yet which amounted in some samples to nearly 2 % of the milk, or 20 % of the solids-not-fat. The existence of this unidentified constituent was discovered by comparing the figures obtained from direct estimation of the solids-not-fat content with those obtained by adding together the protein, lactose and ash. For this purpose the protein content of the milk was taken as 6·38 times the total nitrogen, since the actual protein nitrogen figures were not always available. The lactose was, of course, estimated polarimetrically. The ash was taken as 0·8 %, since on the few occasions on which it was determined the value was found to approximate closely to this figure. The difference values recorded in the penultimate columns of Tables I–III will therefore be subject to slight errors, but such errors are insufficient to invalidate the general conclusion that some hitherto unrecognized substance was present in the milk.

It will be observed that in simple starvation (Table I) the maximum difference between the solids-not-fat and the sum of the protein, lactose and ash is 0·5, the minimum 0·0 and the average for the 12 fasting days 0·3. From other experiments carried out by the authors it appears that the average of 0·3 is a normal figure. But when cow 2 is considered, it will be seen that this value rose from 0·3 to 0·7 on the day when milk fever occurred. It subsequently rose to 1·8 on the sixth and eighth days, after which it decreased again, reaching a normal figure (0·2) by the tenth day of the post-fast period. For cow 3 a fairly normal figure was maintained until the seventh day, when a rise to 1·9 took place. It is significant to observe that this figure was highest in both cows when the lactose was well under 1 % and when the Koestler number was unusually high. Since the lactose content was so low and the percentage content of chloride had not increased sufficiently to maintain the isotonicity of the milk, it appears probable that a substance not usually present in milk in such large amounts was being secreted in quantities sufficient to maintain the usual osmotic pressure. Unfortunately, the presence of this substance was not detected until samples of the milk large enough for detailed analysis and

detection of the unidentified compound were no longer available. There was, however, sufficient milk to redetermine several of the essential figures, and these fully confirmed the earlier results. It is also significant to note that the lactose content was found to be the same whether it was estimated chemically by its reducing power or polarimetrically. This suggests that the unidentified compound was neither a sugar other than lactose nor an optically active water-soluble organic compound. It was thought possible at first that the unknown product might be a glycoprotein, perhaps an intermediary in the preparation of normal milk constituents by the gland as originally suggested by Hammond (6). Such a glycoprotein would have a low nitrogen content, and the factor 6·38 would therefore be too low to give a true estimate of its amount; on the other hand, it would be precipitated during the preparation of the filtrate for sugar analysis, and so would not contribute in any way to the optical rotation of the filtrate. To investigate this point further, some 10 g. of milk protein were prepared from samples in which the unknown substance amounted to 1·8 % of the milk. An examination of the protein itself and of its hydrolytic products failed to reveal the presence of a glycoprotein in the original milk. The work was, however, essentially preliminary in character, and the possibility that the unidentified constituent is a glycoprotein has not been abandoned. In the meantime it is only possible to draw attention to the abnormality and to stress the fact that in the present work it accompanied some abnormal condition in the animals rather than simple inanition.

The existence of this unidentified substance in the milk of cows 2 and 3 suggested that it might also have been present in the milk of animals studied by previous workers, though it had obviously been overlooked. It was therefore decided to calculate the differences between the total solids and the sum of the protein, fat, lactose and ash for all the samples of milk analysed before and during starvation by Overman & Wright (1). The resulting figures are noted with the corresponding lactose contents in Table VI. It will be observed that the differences before the starvation in all three cows are normal (an average of about 0·3), whereas during the fast they exceed 1·0 %, the highest value being 2·4 % for cow 1. It is also of interest that there again appears to be a marked correlation between the increase in this figure and the reduction in the lactose content of the milk. For example, the maximum figures of 2·4, 1·4 and 0·9 are paralleled by lactose figures of 1·50, 2·62 and 3·80 respectively. Overman & Wright do not refer to any illness of their cows during the fast, and it therefore seems possible for the unidentified substance to exist in milk during simple inanition, though this does not tally with the results found in the present investigation. But both the earlier and the present work indicate that the presence of the unidentified substance coincides with an abnormally low lactose content in the milk. As the unknown substance may possibly be an intermediate in the secretion of milk solids, it is obviously desirable that its identity should be established. It is hoped that this will be possible in the near future.

Table VI. *The lactose content of the milk compared with the difference (X) between the total solids content and the sum of the protein, lactose, fat and ash calculated from the results of Overman & Wright*

Cow 1		Cow 2		Cow 3	
Lactose	*X*	Lactose	*X*	Lactose	*X*
		Before inanition			
4·40	0·2	4·45	0·2	4·64	0·5
4·47	0·3	4·56	0·3	4·87	0·3
4·35	0·3	4·50	0·2	4·80	0·4
4·30	0·3	4·45	0·4	4·76	0·3
				4·55	0·6
		Analysis carried out twice daily during the fast			
3·80	0·3	4·15	0·8	4·60	0·0
3·10	1·8	3·68	0·8	3·72	0·5
2·60	1·2	2·82	1·3	3·80	0·9
2·04	1·0	2·77	1·2	3·78	0·6
1·60	1·7	2·62	1·1	3·72	0·7
1·50	2·4	2·62	1·4	3·66	0·8
1·77	1·3	2·70	0·8	3·83	0·8
1·83	2·0	2·55	0·9	3·93	0·7
1·55	1·0			3·79	0·7
1·70	1·1			4·07	0·6

Phosphatase

It has been shown by Folley & Kay (7) and by Folley & White (8) that when the yield of milk and solids-not-fat alters in amount for reasons such as advancement in lactation or administration of thyroxine, the phosphatase content of the milk also changes very markedly, but in the opposite direction. Thus as lactation progresses the amount of this enzyme in the milk increases. They found, however, that this increase is not accompanied by a corresponding increase in serum phosphatase and have therefore suggested that milk phosphatase does not arise from blood phosphatase, but that it is formed by the secreting cells of the mammary gland as a necessary aid in the synthesis of milk constituents. In Table V a few typical values for blood phosphatase are recorded. With cow 1 there was a slight tendency for the values to decrease during inanition, while cow 2 (excluding the day on which the animal suffered from milk fever) the values tended to increase. The differences were, however, slight and of questionable significance. On the other hand, the phosphatase content of the milk of both cows had increased more than fourfold by the sixth day of starvation, the milk yield having simultaneously fallen to a fraction of its initial value. The fact that the phosphatase content of the milk was found to be quite independent of that of the blood is in general agreement with the earlier conclusions of Folley & Kay.

Blood sugar, chloride and corpuscular volume

Although the blood-sugar level is subject to considerable variation as a result of environmental factors (such as undue excitement), the figures given in Table V are sufficiently consistent to provide a clear indication that the

general level tends to rise slightly during the period of fasting.[1] This is directly contrary to the results obtained by Gowen & Tobey (2), who found a fall in both blood and milk sugar during a 3½-day fast. These authors assumed that the fall in the lactose content of the milk which took place during inanition was directly due to a reduction in the blood-sugar level. The present results do not support this view.

The blood-chloride level showed a general tendency towards a reduction of some 10 % in both whole blood and plasma during inanition. Here again no relationship was found between the chloride content of the blood and milk, the latter giving increasingly high values as the fast progressed, i.e. as the milk yield fell. From these and the sugar results, it seems clear that any marked alterations in the secretion of chloride and of lactose in milk are governed by the synthetic activity of the gland and by osmotic relationships and not by the level of the blood constituents.

The corpuscular volume in the blood was found to rise during the fast from about 33 to over 40 %. Presumably the blood was concentrated slightly in order to compensate to some extent for the scarcity of certain blood constituents during inanition.

Total daily yields

In experiments of the type now under discussion it is of interest to consider not only the percentage content of the various constituents in the milk, but also their total daily yields. The figures for cows 2 and 3 cannot be used for this purpose owing to the effect of their abnormal condition on the daily milk yield. The total daily yields for cow 1, where starvation was not accompanied by any abnormal condition, have, however, been recorded in Table VII. It will be seen that before the fast the total daily secretion of solids in the milk of this cow amounted to about 1·99 kg. (1·42 kg. solids-not-fat plus 0·57 kg. fat), but that by the tenth day of the fast the figure had fallen to a minimum of 0·44 kg., returning by the sixteenth day of realimentation to 1·32 kg., or 66 % of the original value.

When each individual constituent is considered separately it is found that the greatest reduction in the amount secreted during the fast took place in the lactose, a daily output of 762 g. falling to only 98 g., or 13 % of the original figure. For fat, protein and solids-not-fat the minimum figures during inanition were 39, 18 and 16 % of their original pre-fast values.

Finally, it may be noted that throughout the 12 days when food was withheld the total excretion of all the solids in the milk of cow 1 was 8·42 kg. At first sight this might appear to represent a very considerable loss to the animal over a 12-day period. Calculation shows, however, that the loss of dry matter actually amounts to not more than 3 % of the total body weight.

[1] With the exception of the sample taken from cow 2 when suffering from milk fever. This animal was treated by the method of udder inflation.

Table VII. *Cow* 1. *Total daily yields of various milk constituents*

Days of fast		Protein* g.	Fat g.	Lactose g.	Solids-not-fat g.	Phosphatase units
Before		485	568	762	1425	5660
During	1	366	478	489	965	6330
	2	209	386	265	562	4040
	3	174	353	241	477	3940
	4	157	358	219	430	4920
	5	148	354	183	370	6450
	6	129	316	153	326	5710
	7	115	303	142	291	4960
	8	112	296	131	280	4180
	9	104	271	109	244	4000
	10	89	220	98	221	3440
	11	93	227	105	223	4100
	12	97	234	114	234	4110
Total during fast		1793	3796	2249	4623	—
After	1	82	182	101	213	2980
	2	99	188	104	227	3620
	3	116	195	117	271	—
	5	189	255	239	486	—
	16	428	292	486	1028	—

* Total nitrogen × 6·38.

Summary

1. The yield and composition of the milk secreted during a 12-day fast is recorded. The solids-not-fat, the nitrogen partition, the chloride, lactose, fat and phosphatase contents are discussed.

2. The presence of an unknown substance in the "abnormal" milk obtained from two of the cows is recorded, and it is shown that similar amounts of an unknown constituent must also have existed in the starvation milk analysed in the course of their work by Overman & Wright(1). The nature of this substance has not yet been elucidated.

3. Certain substances in the blood were estimated before, during and after the fast, and the possibility of relating changes in them with corresponding changes in the milk is discussed.

It is a pleasure to acknowledge the valuable criticism and encouragement received from Dr N. C. Wright throughout the course of the work. Thanks are also extended to Dr A. B. Fowler, Mr S. J. Edwards and Dr S. Morris for assistance with the animals.

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612.664.4:636.2

198. THE DIFFUSION OF TRIMETHYLAMINE OXIDE FROM THE UDDER

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Previous investigations(1) have shown that, in the ruminant, the main tertiary nitrogenous metabolite of betaine and allied tertiary bases is trimethylamine oxide. Experiments showed that on feeding betaine and methyl dimethylamino acetate about 16 % of the nitrogen fed appeared as tertiary nitrogen in the urine. The feeding of choline gave 46 %, while the feeding of trimethylamine and trimethylamine oxide gave quantitative recoveries of nitrogen in the tertiary oxide form in the urine. It was also found that a peak period of excretion occurred at a certain time after feeding, the time taken to reach this point, and the level of maximum excretion, depending on the amount and nature of the tertiary base fed.

It was considered that this short period of maximum excretion in the urine followed closely the period of maximum concentration in the blood. Analysis of milk samples taken periodically after feeding any of the bases also tended to show that the concentration of trimethylamine oxide in milk could be correlated with its concentration in simultaneously drawn samples of urine. It appeared that the oxide was a substance which diffused rapidly through blood and tissue and that, as far as the mammary gland was concerned, there was a constant ratio of concentration of oxide in milk to that in blood.

It was realized that it was the concentration at this peak period which defined the amount of trimethylamine oxide entering the udder. Advice on the feeding of betaine-containing foods, such as beet by-products, to dairy cows therefore centred on keeping the peak excretion period as distant from a milking time as was practically possible(2). When this was done, the possibility of the oxide acting on milk fat and causing the milk to develop a fishy taint was minimized.

In order to consolidate these observations it was necessary to investigate the diffusibility of trimethylamine oxide from the udder into the blood stream and to attempt to account quantitatively for the material in the urine.

Method of experiment

A cow from the Institute's herd was selected and, 1 hr. after the morning milking, a dilute solution of trimethylamine oxide was introduced by means of a teat syphon into two diagonally opposite quarters (L.F. and R.H.) of the udder. Each quarter received 250 mg. of tertiary N. Samples of urine were taken every hour for a 9 hr. period, the urine being quantitatively drawn and collected by the method previously described(1). A control sample of urine

was taken 24 hr. after the end of the experiment. Samples of milk were drawn from individual quarters 3 hr. after introduction of the solutions, and again at the time of evening milking (7 hr. after treatment). A small composite sample from all quarters was taken 4 hr. after treatment.

Methods of analysis

The urine was analysed for tertiary nitrogenous bases as already described by the writer(1).

The milk samples were subjected to complete analysis and the distribution of the volatile base nitrogen also determined. The results for the milk samples are given in Table I and those for the urine samples in Table II.

Table I. *Analysis of milk samples*

Sample	R.H.	L.F.	R.F. + L.H.	Composite	R.H.	L.H.	R.F.	L.F.
Time after treatment (hr.) ...	3	3	3	4	7	7	7	7
% of milk:								
Fat	—	—	—	—	1·70	1·30	1·95	1·00
Total solids	6·78	6·29	11·03	7·10	10·03	10·40	10·54	8·36
Total N	0·479	0·569	0·493	0·510	0·516	0·486	0·466	0·500
Protein N	0·357	0·423	0·479	0·403	0·457	0·468	0·440	0·448
Casein N	0·219	0·118	0·380	0·175	0·320	0·392	0·367	0·290
Non-protein N	0·122	0·146	0·014	0·107	0·059	0·018	0·026	0·052
Cl	0·121	0·228	0·111	0·156	0·143	0·084	0·096	0·125
Ca	0·074	0·061	0·155	0·097	0·126	0·144	0·138	0·113
Total tertiary N	0·086	0·075	nil	0·037	0·007	nil	nil	0·008
NMe_3N	—	—	nil	—	0·0040	—	—	0·0034
NMe_3ON	—	—	nil	—	0·029	—	—	0·0053
% of total N:								
Protein N	74·4	74·4	97·1	80·9	88·6	96·3	94·4	89·8
Casein N	45·7	20·8	77·0	34·4	62·1	80·6	78·7	58·1
Non-protein N	25·6	25·6	2·9	19·1	11·4	3·7	5·6	10·2
Volume ml.	40	40	70	100	450	430	430	500

(Tertiary nitrogen in samples = 177 mg.)

Table II. *Analysis of urine samples*

Wt. of trimethylamine oxide nitrogen introduced into two quarters of the udder = 500 mg.
Depression of freezing point of the solution injected = 0·689°

Sample no.	Time after treatment hr.	Volume l.	Total NMe_3N mg.	NMe_3ON mg. per 100 ml.	NMe_3ON Total mg.
1 (control)	0	(0·51)	nil	0·08	(0·4)
2	1	0·41	4·8	2·38	9·8
3	2	0·26	1·4	5·25	13·7
4	3	0·44	3·5	11·82	52·0
5	4·3	0·65	2·6	9·85	64·0
6	5·3	1·36	5·4	2·38	32·4
7	6·3	3·47	9·7	0·88	30·5
8	8·3	0·42	2·9	2·70	11·3
9 (control)	24	(0·15)	1·0	1·85	—
Total		7·01	31·3		213·7

Total recovery of tertiary nitrogen in urine = 245 mg. (= 49 %)
Tertiary nitrogen in milk samples = 177 mg.
Total nitrogen accounted for = 422 mg. (= 84 %)
Ratio of NMe_3O nitrogen : NMe_3 nitrogen = 7·0.

Discussion of results

About an hour after treatment the injected quarters became turgid and the turgidity increased up to 2½ hr. The untreated quarters were flaccid. Three hours after injection the turgidity relaxed and samples sufficient only for analysis were taken from all quarters. Four hours after injection, milking was difficult and the amounts obtained from each quarter were bulked for analysis. Milking at the usual evening milking time gave no difficulty and a total yield of 1·8 l. was obtained.

The cause of the turgidity was undoubtedly the dilution of the fluid in the quarter; in one quarter (L.F.) there was evidence of the entry of a considerable amount of sodium chloride, which however had decreased almost to the normal value for milk in 7 hr. In spite of the dilution the concentration of trimethylamine oxide solution after 3 hr. was still 0·42 % (0·08 % tertiary N) but, in 7 hr., the concentration had decreased to 0·008 % of tertiary N. It was obvious that the condition of turgidity was not favourable to a high rate of diffusion from the quarter into the blood. It seems that the first step in the process was to make the contents of the quarter isotonic with the ordinary mammary secretion, and this was followed by a relief of the pressure in the quarter by refiltration of liquid into the blood. The concentration of trimethylamine oxide in the urine bears this out, since the maximum concentration immediately followed the period of maximum turgidity of the quarter (3–4 hr. after treatment). The largest excretion of the base took place 4–6 hr. after treatment, and was accompanied by increasing diuresis. About 35 % (or 70 % of the total excretion) of the trimethylamine oxide occurred within 4·3 hr. of its introduction into the quarters, irrespective of the osmotic complications arising from using a solution of higher osmotic pressure and from prolonged turgidity of the quarters.

The analysis of the milk from the non-injected quarters showed that under the conditions of this experiment none of the tertiary base entered them. The amount of milk which could be obtained from these quarters 4 hr. after the morning milking was very small, and it seems probable that the conditions in the treated quarters must have affected milk secretion in the other two. The protein distribution of the composite sample taken at this time was normal but the total solids content was low. This, however, was also true for both quarters at the evening milking.

The effect on the treated quarters had not disappeared 7 hr. after treatment. The protein distribution was still abnormal. The non-protein nitrogen, instead of being 5–6 %, was 10–11 %. The total nitrogen content was comparatively low, and casein nitrogen accounted for only 58–62 % instead of 76–77 %. The chloride content of the milk from the untreated quarters was normal in value, but that of the milk from the treated quarters was too high. The calcium content was also much lower than that of the milk of the untreated quarters.

The full analysis of the milk brings out the disturbance in composition resulting from interference with udder function in the present experiment.

The ratio of free trimethylamine nitrogen to trimethylamine oxide nitrogen in the urine was of the same order as was found in the earlier metabolism experiments(1). None was found in the control sample of urine taken at the start of the experimental period and a sample taken 24 hr. afterwards contained only a trace.

Of the 500 mg. of tertiary nitrogen introduced into the two quarters, 177 mg. were removed from the udder in the milk samples; this left 323 mg. for diffusion. In 8·3 hr., a total of 245 mg. of tertiary nitrogen was recovered from the urine, representing a yield of 76 %. The deficiency can be accounted for by the fact that all the urinary tertiary nitrogen had not been recovered, since the last sample taken contained nearly 2 mg. per 100 ml. In spite of the physiological disturbances associated with this type of experiment, it is believed that satisfactory proof has been obtained of the rapid diffusion of trimethylamine oxide from the udder to the blood stream and its later excretion in the urine.

Summary

On injecting a neutral solution of pure trimethylamine oxide into two quarters of an udder, the quarters increased in turgidity up to 2·5 hr. and then relaxed. The oxide diffused rapidly out of the quarters during the third and fourth hours after injection and a maximum concentration was shown in the urine at the end of the third hour. In a period of 8·3 hr., 76 % of the oxide available for diffusion was recovered from the urine.

The milk obtained 7 hr. after injection contained 7–8 mg. % of tertiary nitrogen, but the secretion had not in that time regained normal composition owing to the interference with the natural functioning of the quarters.

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199. THE CHLORIDE CONTENT OF MILK

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(With 3 Figures)

The importance of the chloride content of milk as a "single-value" property has already been discussed by the writer(1, 2). The complementary relationship between lactose and chloride in milk has been recognized for over 40 years, and the calculation of the approximate lactose content of milk from the chloride content by Mathieu & Ferré's(3) formula: (Lactose (g. per l.) + 12·9 × sodium chloride (g. per l.) = 70) is of considerable value especially in assessing the part played by low lactose content in causing milk to be low in solids-not-fat.

If, again, milk secretion be regarded, as far as the truly soluble constituents are concerned, as capable of replacing the sodium chloride of blood serum by lactose, the chloride content of milk is a criterion of the performance of the cow in this respect. A low chloride content denotes a high level of lactose secretion. There is also a negative correlation between chloride and solids-not-fat content(4). For all conditions found responsible for deficiency of solids-not-fat in milk, the chloride content is higher than the value for normal milk and is especially so with milk from cows with severe udder infections and at the end of lactation(5). In many cases of mild mastitis, however, there is only a slight rise in chloride content.

The sodium content can be accurately calculated from the chloride content of milk by using the equation Cl (mg. %) = Na (mg. %) × 1·24 + 18·1(6). These authors found that when variations in chloride content occurred, the cation which varied in a parallel manner was sodium.

Scope of the Investigation

It is well known that the chloride contents of samples of milk from individual cows show great variations. This paper deals with the magnitude and frequency of such variations. The variations depend on a variety of factors such as breed and age of cow, stage of lactation, season of year, weather conditions and health of the udder. The effects of some of these conditions on chloride content have been studied.

The chloride content of the milk from individual cows in the Institute herd of Shorthorns and Guernseys has been determined over a period of more than 3 years. A similar study has been made over two winter periods in a herd of Friesians in Hampshire which gave milk of low quality and in a small Ayrshire herd. The results of a large number of samples which have been examined

during advisory and experimental work are also available. In all, the results for over 7800 samples of milk are summarized in this paper.

Methods of determination of chloride content

The method used for chloride determination was a modification of that described by the writer(1). This is an open Carius method in which 10 ml. of milk are digested with 10 ml. of concentrated nitric acid after the addition of excess of standard silver nitrate solution until all the casein is dissolved and reddish brown fumes are evolved. The excess of silver nitrate is then titrated with standard thiocyanate using as indicator 1 ml. of saturated iron alum in 10 % nitric acid (Volhard method). No permanganate is necessary to aid digestion of organic matter in milk and the addition of urea to remove nitrous acid is unnecessary. Other methods have been tried but found unsatisfactory either from the points of view of accuracy or speed of working. The direct titration method suggested by Rosell(7) gave too high results owing to combination of silver ions with protein. Methods depending on titration of the chloride in a clear serum of milk were found to be comparatively slow and subject to error, since no correction (or at least a very approximate correction) is made in such methods for the volume of the precipitate. The acid digestion method was found to be reliable and expeditious, up to thirty samples per hour being dealt with.

Results

In view of the very large number of results available, it was decided to tabulate the frequency distributions only. Table I shows the frequency distributions for morning and evening samples of the Institute herd for each of the years 1934–6, the total for the 3 years and the percentage of the samples falling into each range of chloride content.

Table II shows the distribution of the values obtained for morning and evening samples for Guernsey, Shorthorn and a few Ayrshire cows, and the percentage distribution for the first two breeds. These results cover a period of 3 years, sampling being done from about thirty Guernsey and fifty Shorthorn cows. Table III shows the results obtained for samples of milk obtained from Friesian herds in connexion with an inquiry into the cause of low solids-not-fat in the milk from these herds(8). The table also contains the frequency distribution of samples of morning and evening milk from all sources and the percentage distribution. The results for the Friesian samples are not included in these columns. The distribution for 100 samples of bulked milk from the Institute herd are also given.

The frequency distribution curves for the three years' samples from the Institute herd, for the Shorthorn, Guernsey and Friesian samples and for all samples are given in Figs. 1 and 2.

Table I. *Frequency distribution of the chloride content of the milk of individual cows of the Institute herd for the years* 1934–6 (Cl *as mg.* %)

Year ...	1934		1935		1936		1934–6			Percentage of
Cl	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	Total	samples
– 40	0	0	1	1	0	0	1	1	2	—
– 50	2	0	7	7	2	3	11	10	21	0·7
– 60	12	15	24	29	24	36	60	80	140	4·8
– 70	44	47	56	82	77	73	177	202	379	13·0
– 80	49	70	103	98	96	117	248	285	533	18·3
– 90	86	68	73	48	99	97	258	213	471	16·1
– 100	66	63	54	50	75	61	195	174	369	12·7
– 110	45	49	38	44	43	37	126	130	256	8·8
– 120	34	34	48	30	31	37	113	101	214	7·3
– 130	43	33	20	16	32	24	95	73	168	5·8
– 140	22	16	18	19	18	14	58	49	107	3·7
– 150	15	4	21	14	21	19	57	37	94	3·2
– 160	12	9	8	9	8	2	28	20	48	1·6
– 170	3	7	17	6	1	2	21	15	36	1·2
– 180	1	3	3	3	3	3	7	9	16	0·5
– 190	2	2	3	0	3	2	8	4	12	0·4
– 200	5	1	3	1	5	2	13	4	17	0·7
– 210	4	3	0	1	2	2	6	6	12	0·4
– 220	2	0	1	1	2	1	5	2	7	0·2
– 230	0	0	0	0	1	1	1	1	2	0·1
– 240	1	1	0	0	4	0	5	1	6	0·2
– 250	0	1	0	0	0	2	0	3	3	0·1
– 260	0	1	2	0	0	0	2	1	3	0·1
– 270	0	0	1	0	1	0	2	0	2	—
No. of samples	448	427	501	459	548	535	1497	1421	2918	

N.B. In all tables each chloride group differs consecutively by 10 mg. Each group is indicated by a *minus* sign before the maximum chloride figure in that group, e.g. – 120 is the group containing 111–120 mg. %.

Table II. *Frequency distribution of chloride contents of milk of individual Guernsey, Shorthorn and Ayrshire cows* (Cl *as mg.* %)

	Guernsey				Shorthorn				Ayrshire		
Cl	a.m.	p.m.	Total	%	a.m.	p.m.	Total	%	a.m.	p.m.	Total
– 40	0	0	0	—	1	1	2	0·1	0	0	—
– 50	5	5	10	1·0	6	5	11	0·5	0	0	—
– 60	13	21	34	3·5	48	61	109	5·4	0	0	—
– 70	56	79	135	13·9	123	125	248	12·3	4	4	8
– 80	94	106	200	20·7	160	189	349	17·3	2	4	6
– 90	90	78	168	17·4	178	141	319	15·8	2	3	5
– 100	55	55	110	11·4	148	128	276	13·7	9	7	16
– 110	39	36	75	7·7	92	95	187	9·3	12	8	20
– 120	30	45	75	7·7	86	59	145	7·2	4	10	14
– 130	34	25	59	6·1	61	50	111	5·5	8	3	11
– 140	16	17	33	3·4	42	32	74	3·6	1	2	3
– 150	16	10	26	2·7	41	27	68	3·4	1	1	2
– 160	11	9	20	2·1	17	11	28	1·4			
– 170	9	4	13	1·3	12	11	23	0·9			
– 180	1	1	2	0·2	6	8	14	0·7			
– 190	0	0	0	—	8	4	12	0·6			
– 200	2	0	2	0·2	11	4	15	0·7			
– 210	2	1	3	0·3	4	5	9	0·4			
– 220	1	1	2	0·2	4	1	5	0·3			
– 230	1	0	1	0·1	0	1	1	—			
– 240	0	0	0	—	5	1	6	0·3			
– 250	0	0	0	—	0	3	3	0·2			
– 260	0	0	0	—	2	1	3	0·2			
– 270	0	0	0	—	2	0	2	0·1			
Total no. of samples	475	493	968	99·9	1057	963	2020	99·9	43	42	85

Table III. *Frequency distribution of chloride content of milk from Friesian cows, for all other samples, and for bulk milk of Institute herd (a.m.)* (Cl *as mg.* %)

	Friesian (special herds)				All samples except Friesian				Bulk milk
Cl	a.m.	p.m.	Total	%	a.m.	p.m.	Total	%	a.m.
– 40	0	0	0	—	6	1	7	0·1	—
– 50	0	0	0	—	7	10	17	0·3	—
– 60	0	0	0	—	67	94	161	2·6	—
– 70	8	11	19	1·2	263	288	551	9·1	7
– 80	22	19	41	2·5	540	515	1055	17·4	33
– 90	47	50	97	6·0	673	401	1074	17·7	28
–100	58	60	118	7·2	469	325	794	13·1	22
–110	78	103	181	11·1	370	282	652	10·7	5
–120	114	126	240	14·8	378	296	674	11·1	5
–130	129	104	233	14·3	207	171	378	6·2	—
–140	117	91	208	12·8	123	95	218	3·6	—
–150	83	61	144	8·9	98	62	160	2·6	—
–160	68	64	132	8·1	48	35	83	1·4	—
–170	32	29	61	3·7	48	29	77	1·3	—
–180	30	26	56	3·4	22	22	44	0·7	—
–190	31	12	43	2·6	20	17	37	0·6	—
–200	10	10	20	1·2	16	8	24	0·4	—
–210	7	6	13	0·8	7	9	16	0·3	—
–220	5	2	7	0·4	7	5	12	0·2	—
–230	3	2	5	0·3	8	4	12	0·2	—
–240	3	0	3	0·2	8	6	14	0·2	—
–250	2	2	4	0·2	3	3	6	0·1	—
–260	0	0	0	—	3	3	6	0·1	—
–270	0	0	0	—	2	2	4	—	—
Total no. of samples	847	778	1625	99·7	3393	2683	6076	100·0	100

Discussion of results

(*a*) *Mean chloride content.* The mean chloride contents of the samples are given in Table IV. For comparison it may be stated that the mean chloride content of 100 bulk morning samples from the Institute herd, collected over a period of 2 years, was 85 mg. %. Mean figures are, however, of doubtful significance, since the inclusion of a small number of samples taken at the end of lactation will tend to raise the mean unduly. In bulk milk, on the other hand, the volume accounted for by end of lactation milk is small, and mixed herd milk will therefore usually have a lower chloride content than the unweighted mean calculated from that of the individual samples.

Table IV. *Mean chloride content of milk samples* (Cl *as mg.* %)

	Institute herd							
	1934	1935	1936	1934–6	Guernsey	Shorthorn	All samples	Friesian
A.m.	97	96	92	94	95	98	99	132
P.m.	93	91	91	92	91	96	97	126
All samples	95	94	92	93	93	97	98	128

Caulfield & Riddell(9) give the following average chloride contents (mg. %) for the milks of different breeds: Guernsey 124, Jersey 125, Ayrshire 126, and Holstein (Friesian) 139 mg. %. In comparison with the present results these values are excessively high. They report, however, that 96 % of 1006 samples contained between 80 and 120 mg. %.

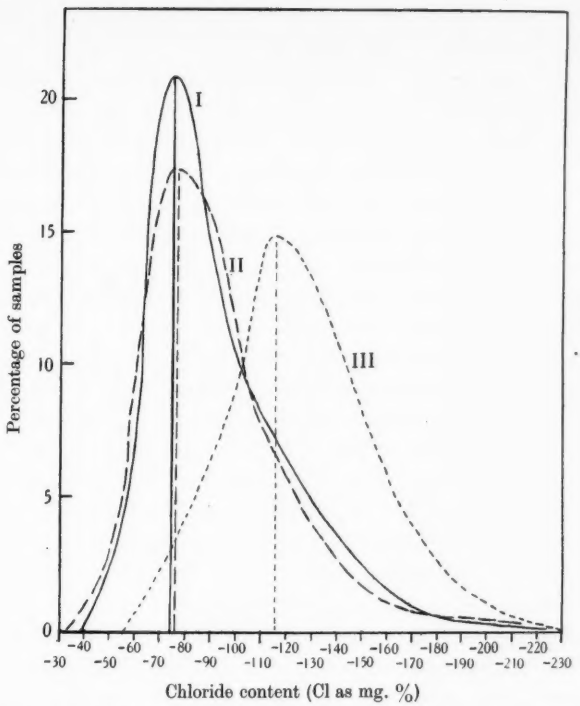

Fig. 1. Frequency distribution of individual samples of milk from I, Guernsey cows (968 samples); II, Shorthorn cows (2020 samples); III, Friesian cows, special herds (1625 samples).

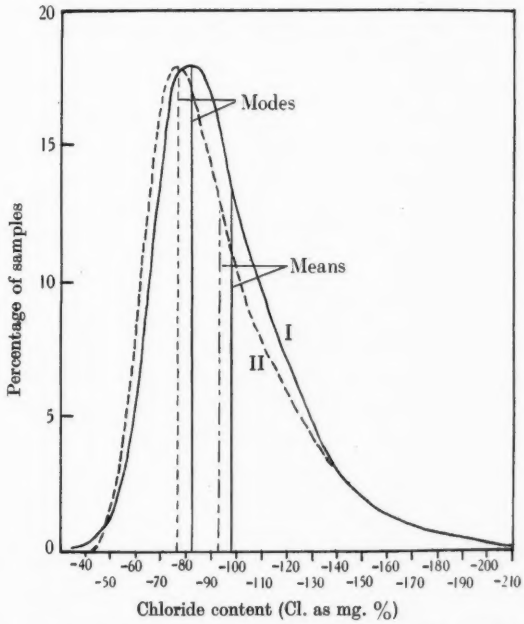

Fig. 2. Frequency distribution of chloride content of individual milk samples from I, All samples except Friesian (1076 samples); II, Samples from Institute herd (2931 samples).

(*b*) *Frequency distribution.* The *modes* of the frequency distributions lie in the 71–80 range (mg. Cl %) for Guernsey, Shorthorn and the Institute herd milk. For samples from the special Friesian herds the mode is in the range 111–120, and for the large group comprising all samples between 81 and 90. In Fig. 2 it can be seen that, owing to the weighting of the average chloride content by a larger number of samples on the right of the modes, the means are considerably higher than the modes. All the distribution curves show a preponderance of samples to the right of the mode, the frequency decreasing gradually until over 200 mg. % is reached.

The *modal range*[1] of distribution lies between 55 and 120 mg. %. Over 80 % of Guernsey, Shorthorn and Institute herd milk and 92 % of all samples except Friesian fall between these values, while 73 % of Friesian samples fall between the values 85 and 150 mg. %.

Evening milk consistently has a lower chloride content than morning milk. This amounts on the average to about 5 mg. % less in Guernsey, and 8 mg. % less in Shorthorn and Friesian milk. The same difference is brought out in the large sample group. The morning milk has a chloride content most frequently between 81 and 90 and the evening milk between 71 and 80.

The samples outside the modal ranges for each group are greater for the high values of chloride (above 120) than for values below 55 mg. %. Milk samples at end of lactation and from cows persistently giving high chloride milk are far more numerous than those from cows giving low chloride milk, the last group consisting chiefly of newly calved heifers. The lowest value found was 34, and the highest 294 mg. %.

(*c*) *Breed of cow.* There was no great difference between the distributions of the chloride content of Shorthorn and Guernsey milk. There was a slightly greater percentage of the latter samples at the modal value. Ayrshire milk contained roughly 10 mg. % more chloride than Shorthorn and Guernsey milk. The value for the Friesian milk included in this survey was about 40 mg. % higher, but these samples must not be taken as representative of Friesian milk generally. The samples of all breeds taken together (6076 samples of morning and evening milk) show a mode of about 83 mg. % of chloride.

(*d*) *Age of cow.* On the whole, milk from successive lactations of a cow increases in chloride content. All the values under 40, and most of those under 50 mg. % were for milk from heifers early in their first lactation. The majority of samples were from cows in their second to fifth lactations and contained chloride from 60 to 100 mg. %. Aged cows gave milk of still higher chloride content. One Guernsey cow, for instance, gave milk containing an average of 0·145 % Cl throughout three successive lactations. An aged Guernsey (29 years) gave an abnormal secretion with 195 mg. % of chloride.

(*e*) *Season of year.* Periods of drought in summer which were accompanied

[1] This term is used to describe the close range on each side of the mode within which roughly two-thirds of the samples fall. No quantitative significance is ascribed to the expression.

by a decrease in the solids-not-fat content, cause an appreciable increase in the chloride content of milk. The ten bulk samples containing over 100 mg. % of chloride reported in Table III were taken from the bulked milk of the Institute herd during the drought of July–September 1933. Lactational effects, of course, also influenced seasonal variations, but on the whole the effect of changing from winter feeding to spring grass is to lower the chloride content. This was especially marked with the Friesian herds which were giving milk of low solids-not-fat content. After the winter-feeding period of 1930–1, during which 81 % of 1221 samples were found to contain less than 8·5 % of solids-not-fat, the lowering of chloride content and rise in solids-not-fat on turning out to grass was phenomenal. Thus evening samples taken on 24 March contained an average of 142 and those on 11 May 105 mg. % of chloride. The effect of turning out to grass in the next season, when only 65 % of 682 samples were below 8·5 % in solids-not-fat content, was less marked. The effect of other environmental factors have not been studied, as in most cases the periods between samplings (1 month) did not justify any deductions being made.

(*f*) *Lactation period.* Inspection of the monthly values shows that the chloride content increased only slowly during the period when milk yield is relatively high. At the end of this period, usually after the seventh month, the chloride content rose rapidly until at the beginning of the period of drying off, very high values were found. The chloride content of milk after the colostral period was variable and might be either above or below the average for the lactation. The milk of cows in good health was found to be very regular in chloride content whether the period of sampling was monthly, weekly or daily. Cows which gave milk high in chloride either as an individual characteristic, or because of drought or udder infections showed considerable variation in the amount of chloride they secreted in their milk when sampled daily or weekly.

Although the chloride content rose towards the end of lactation the total amount of chloride secreted decreased with advancing lactation, owing to the marked reduction in total yield of milk. In full flush Friesian cows secreted 11–12 g. of chloride in a morning milking, but this fell to 5·7 g. at a later stage of lactation, although a considerable rise in chloride content of the milk had occurred.

(*g*) *Other factors.* Udder infection in the advanced stage caused a considerable increase in chloride content of milk, but only small but significant increases were evident with slight inflammation, i.e. in cases where the milk did not appear abnormal.

Relation between chloride content and electrical conductivity

The electrical conductivity at 18° C. of over 100 samples of milk of chloride content ranging from 61 to 235 mg. % was determined by the bridge method. No difficulty was obtained in getting sharp null points with all milk samples when the electrode surfaces were properly prepared.

A scatter diagram illustrating the relationship between conductivity and chloride content of samples over a wide range of values is shown in Fig. 3. It will be seen that conductivity tends to increase with chloride content, the values being evenly scattered about the line expressed by conductivity (mhos $\times 10^4$) = 27·5 + 0·175 × Cl (mg. %). The 102 samples examined had a mean of 110 mg. % of chloride and a conductivity of 46·4 $\times 10^4$ mhos. About 83 % had a chloride content within the range of 60–130 mg. % and 80 % showed

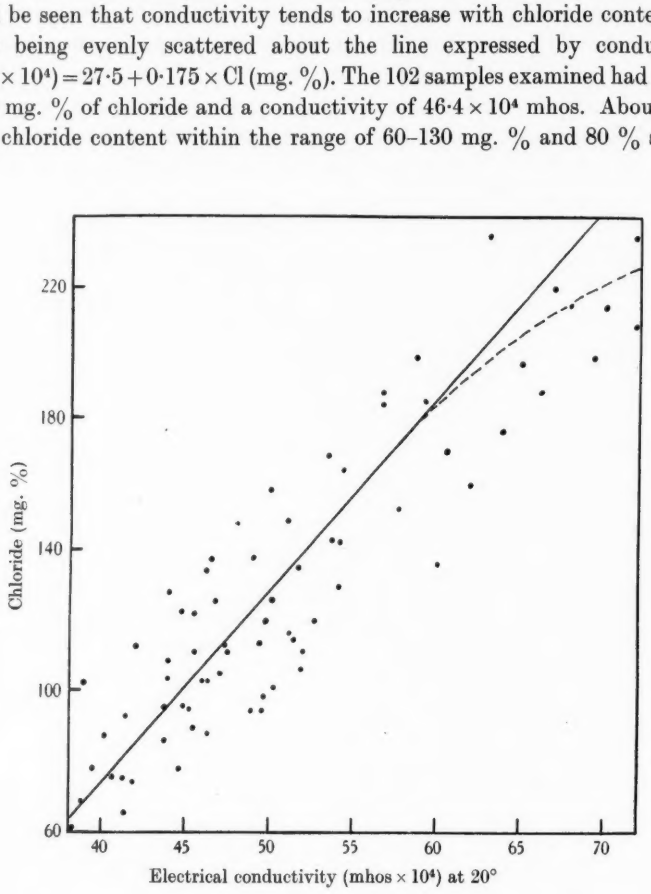

Fig. 3. Scatter diagram showing relationship between electrical conductivity and chloride content.

a conductivity between 40 and 54 $\times 10^{-4}$ mhos. Both the means and the ranges for normal milk agreed with values obtained by Koeppe(10) and Plucker & Steinruck(11) for continental milk.

In samples of high chloride content and high conductivity there was a tendency for the latter to increase at a greater rate than the former. This is possibly due to the higher concentration of electrolytes other than chloride in such abnormal milk, e.g. non-protein nitrogenous substances. The lower viscosity of such milk also indicates a higher ionic mobility.

SUMMARY

The frequency distribution of the chloride contents of 7802 samples of milk, grouped into morning and evening samples, monthly samples for three consecutive years from the Institute herd, samples from Shorthorn, Guernsey, Ayrshire and Friesian cows, and other miscellaneous samples, has been determined. The *mode* for morning milk is 81–90 and for evening milk 71–80 mg. %. The *mode* for Guernsey and Shorthorn milk is 71–80 and for all (6076) samples, 81–90 mg. %. The *mode* for milk from herds of Friesians known to give milk of low content of solids-not-fat was 111–120 mg. %, but this must not be taken as the value for this breed generally. The average for Shorthorn milk is 97, Guernsey 93, Institute herd 93, all samples 98, special Friesian herd 128 and bulk Institute milk 85 mg. %.

Factors influencing the chloride content of milk such as breed, age of cow, lactation period, number of lactations, seasonal effects, and udder troubles have been discussed from inspection and by deduction from the series of values obtained.

The electrical conductivity of milk increases with chloride content but there is some deviation from the linear law. The conductivity increases at a greater rate than the chloride content in samples high in chloride, probably owing to the increase in electrolytes other than chloride.

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637.127.8+637.344:545.371

200. THE COLORIMETRIC DETERMINATION OF *p*H IN MILK AND WHEY BY MEANS OF THE "WULFF" *p*H TESTER

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It has been repeatedly pointed out (1, 2, 3) that the properties of cheese during the different stages of its manufacture should be correlated with the hydrogen-ion concentration rather than with the titratable acidity. Little systematic work has, however, so far been carried out in this direction, except for a study of the relationship between *p*H and titratable acidity in Cheddar cheese by Brown & Price (4). In planning work on similar lines, it was realized that the potentiometric methods of determining *p*H require expensive equipment and skilled attention, so that a supplementary colorimetric method, if sufficiently accurate to indicate the major changes in *p*H, should appeal more strongly to the practical cheesemaker on account of its cheapness and simplicity and the ease with which the outfit can be transported.

In applying colorimetric tests to milk and whey, difficulties are caused by the turbidity and colour of these liquids. In order to overcome these difficulties various methods which, however, complicate the technique of the tests have been proposed. It therefore seemed of interest to investigate the applicability of the "Wulff" *p*H Tester. Its possible use in dairy work has been indicated by Karsten (5). The principle of the tester, viz. to insert strips of a water-diffusible membrane containing indicators into the liquid to be tested and to compare the colour of the strips with standard colours mounted on a sliding scale, makes the test particularly suitable for determinations in which turbidity and colour are apt to interfere with methods based on the direct comparison of liquids. The technique is very simple and a determination can be completed in 2–3 min.

Experimental

Using the colour standard II (*p*H 5·0–7·2) and the corresponding indicator strips of the "Wulff" *p*H Tester, a number of milks and wheys were examined. Wheys were prepared from normal milks and also from the milk of cows known to be badly infected with mastitis in order to see whether such abnormal wheys could be tested with equal accuracy. Furthermore, some wheys were artificially soured by adding lactic acid, in order to cover the total range of *p*H normally encountered in whey during cheesemaking. The best results were obtained when the indicator strips were inserted for 1½ min. and rinsed with distilled water before comparison. Tables I–III contain the results of colorimetric and potentiometric *p*H determinations, the latter being carried out by an independent observer.

Table I. *p*H *of milk*

No.		*p*H "Wulff"	*p*H Glass electrode	Difference
1	Bulk milk	6·8	6·76	+0·04
2		6·7	6·74	−0·04
3		6·7	6·78	−0·08
4		6·7	6·72	−0·02
5	Individual cow's milk	6·9	6·76	+0·14
6		6·5	6·66	−0·16
7		6·7	6·83	−0·13
8		6·5	6·58	−0·08

Table II. *p*H *of whey*

No.		*p*H "Wulff"	*p*H Glass electrode	Difference
1	From normal milk	6·7	6·67	+0·03
2		6·5	6·48	+0·02
3		6·7	6·82	−0·12
4		6·6	6·61	−0·01
5		6·8	6·72	+0·08
6		6·5	6·62	−0·12
7	From mastitis milk	6·7	6·82	−0·12
8		7·0	6·94	+0·06
9		7·1	7·04	+0·06
10		5·5	5·60	−0·10
11		6·3	6·42	−0·12
12		6·8	6·80	±0

Table III. *p*H *of artificially soured whey*

"Wulff"	6·5	6·1	5·8	5·3	6·5	6·0
Glass electrode	6·48	6·04	5·74	5·28	6·62	6·03
Difference	+0·02	+0·06	+0·06	+0·02	−0·12	−0·03

DISCUSSION OF RESULTS

The tables show that for whey the agreement between the colorimetric and potentiometric measurements is as close as can be expected from any colorimetric method, viz. about 0·1 *p*H unit. Slightly inferior results were obtained with milk.

The figures given by Brown & Price(4) show that during the initial stages of cheesemaking the changes in *p*H are so small that they can be detected only by sensitive potentiometric methods and it seems doubtful whether any significant relationship between *p*H and the properties of cheese can be established during these stages. Since, in any case, no colorimetric test can be employed for this purpose, it matters little that the *p*H of the original milk cannot be determined by the "Wulff" *p*H Tester quite as accurately as desired. As the *p*H of whey can be measured with greater accuracy, the method should be helpful in following the more definite changes in *p*H which occur after cutting the curd. It is for the later stages that there seems better

prospect of successfully correlating the properties of cheese with *p*H instead of with the titratable acidity.

The author's thanks are due to Mr H. Hallett who carried out the potentiometric determinations.

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637.321

201. THE SOLUBILITY OF CHEDDAR CHEESE CURD IN SODIUM CHLORIDE SOLUTIONS

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The importance of the concentration of salt in the aqueous portion of Cheddar cheese has been demonstrated by Riddet *et al.* (1), who showed that cheese of good quality contained on the average 1·66 % of salt, equivalent to a concentration of 4·8 % salt in the moisture of the cheese. These findings have recently been confirmed by Marquardt (2) for American Cheddar cheese. McDowall & Whelan (3) showed that the growth of lactic streptococci in milk is largely inhibited by a 5 % concentration of salt, and suggested a possible relationship between this inhibition of bacterial growth and the effect on the cheese quality of the salt concentration in the water of the cheeses. The relation of salt concentration to curd solubility seemed also to be a possible contributory cause.

Sammis & Hart (4) and Van Slyke & Bosworth (5) have shown that the proteins of cheese are soluble in solutions of sodium, potassium, magnesium and ammonium chlorides, but have not defined the variation in salt solubility of the proteins in cheese at different periods after manufacture.

Experimental

Manufacture of cheese. Milk drawn from the same milk supply was run into two small vats. Coagulation was obtained by addition of rennet in the proportions of 3 and 5 oz. respectively per 1000 lb. of milk. The manufacturing conditions were otherwise normal and similar for the two vats. From each vat a number of loaf cheese were made. The cheese were held at 60° F. for 14 days, and thereafter in a cool room at 45–50° F.

Determinations of soluble nitrogen. At intervals throughout the ripening period of the cheese, determinations of the solubility of the proteins in water and in sodium chloride solutions were made according to the following procedure. Representative samples (100 g.) were ground to a homogeneous paste in a mortar. Moisture and total nitrogen estimations were made on a portion. Duplicate 5 g. samples of the remainder were ground in a mortar (40 ml. capacity) for 2 min. at 50° C. with 10 ml. water, or 1, 3, 4, 7, 10 % sodium chloride solutions. The mortar was warmed before commencement of the grinding and during grinding was held in a bath of water at 50° C. The pasty mass was washed into a 100 ml. graduated flask with the water or appropriate salt solution at 50° C. The flasks were filled to the mark, shaken, and then allowed to stand in an incubator at 35° C. for 90 min. Fat was removed by

suction, the residue was poured on to a fluted filter paper and left overnight. Nitrogen determinations were made in duplicate on the filtrates. The results for the duplicate grindings with each solution were in good agreement throughout.

Results

The results, expressed as the percentage of the total nitrogen in cheese found to be soluble in the respective solutions, are given in Tables I and II. It is evident that within 10 days of the time of manufacture of Cheddar cheese the protein material becomes almost completely soluble in sodium chloride brines of any concentration within the range 3–10 %. The solubility in a 1 %

Table I. *Normal rennet addition*

Age of cheese days	Moisture content of cheese %	Nitrogen in cheese (expressed as a percentage of the total nitrogen content) soluble in brines of the following concentrations						
		0 %	1 %	3 %	4 %	5 %	7 %	10 %
0	40·1	4·3	6·0	12·9	44·6	35·7	39·7	44·3
1	35·8	5·3	5·9	21·4	35·8	37·9	57·1	53·4
2	34·5	5·1	7·2	57·0	57·2	70·9	76·4	71·1
4	35·0	4·4	8·4	49·2	71·1	86·3	87·4	86·1
7	34·6	6·9	16·0	94·2	96·6	97·4	98·4	99·7
10	33·8	7·1	16·7	94·1	97·7	100·0	100·1	100·0
15	33·9	9·3	16·7	88·2	98·2	99·0	97·2	98·7
21	32·3	13·4	20·4	97·4	98·2	100·8	100·5	98·7
24	31·5	16·6	21·8	97·7	100·0	100·0	100·7	99·5
28	34·2	11·9	26·4	101·4	101·6	100·8	101·4	101·1
35	34·7	13·1	24·5	101·7	101·5	102·3	101·7	102·0
42	33·7	13·4	18·4	101·8	101·6	101·0	101·3	99·7
56	32·9	14·2	23·5	101·3	—	—	—	—
77	33·0	16·3	20·8	99·0	99·7	100·0	100·3	100·5
98	32·2	18·1	21·9	99·8	99·0	99·0	99·0	93·6
142	30·1	21·8	25·0	98·0	98·5	98·8	98·8	97·8
178	30·9	24·2	26·7	100·0	99·8	99·2	100·0	—
238	27·5	23·7	25·9	91·7	101·7	101·0	99·0	—
257	27·0	24·9	27·9	94·4	97·0	98·4	95·4	—

Table II. *High rennet addition*

Age of cheese days	Moisture content of cheese %	Nitrogen in cheese (expressed as a percentage of the total nitrogen content) soluble in brines of the following concentrations						
		0 %	1 %	3 %	4 %	5 %	7 %	10 %
0	40·0	4·2	4·5	13·9	35·9	41·6	39·1	52·6
1	35·5	4·3	5·6	28·2	33·6	31·2	53·2	67·7
2	34·9	5·4	6·6	47·8	70·2	67·0	87·1	72·9
4	35·5	6·1	6·9	44·3	81·1	81·4	92·5	84·5
7	34·8	7·7	19·8	95·0	98·6	97·1	96·3	98·2
10	34·8	8·8	18·7	96·4	99·7	100·1	101·2	98·7
15	34·3	9·6	16·3	98·7	99·5	98·7	99·5	99·1
21	34·0	12·9	21·9	96·1	98·2	106·7	99·2	97·9
24	33·0	13·0	29·9	100·1	100·9	102·2	101·4	100·4
28	33·6	15·4	23·6	101·0	101·2	101·8	101·5	101·5
35	34·1	16·0	23·6	99·8	99·6	100·4	101·4	100·4
42	33·7	17·5	21·5	101·6	102·4	102·1	101·3	100·7
56	32·6	18·4	26·2	—	—	—	—	—
77	32·5	20·5	24·9	99·4	100·0	100·0	101·0	100·0
98	32·0	22·8	27·7	99·8	100·0	99·8	100·5	94·5
142	30·0	27·5	30·9	97·8	97·8	98·3	98·3	98·3
178	30·7	29·2	31·2	98·2	99·0	98·5	99·8	—
238	27·5	27·6	28·8	99·9	90·9	98·3	100·6	—
257	27·1	29·4	23·4	94·4	97·0	97·9	97·7	—

sodium chloride brine was slightly greater than the solubility in distilled water, but not markedly so.

There was little difference in the brine solubilities of the cheese from milk treated with the normal and the high proportion of rennet. The difference in water solubility of the cheese made with the high proportion of rennet began to appear quite early in the life of the cheese. After 40–50 days this difference remained approximately constant with increasing age of the cheese up to 8 months. This observation is also in accord with previous results (1). The change of solubility of cheese proteins in water with increase in age of the cheese is similar to that recorded previously at this Institute (Riddet *et al.* (1), p. 32).

Summary and conclusions

Measurements are reported of the solubility, in water and in sodium chloride solution of different concentrations, of the protein of cheese at various times after manufacture. The solubility in 3–10 % sodium chloride solutions reached approximately 100 % within 7 days after manufacture of the cheese, both for normal and high rennet cheese, and remained at that value throughout the life of the cheese (8 months). Since the proteins of cheese are thus shown to be equally soluble in sodium chloride solutions corresponding in concentration to those encountered in the whole range of commercial cheese (1), the effect of salt concentration on cheese quality is not to be attributed to a variation in the solubility of the protein in the brine.

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637.322

202. THE COMPOSITION OF CHEESE RINDS

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In the manufacture of processed cheese, the rinds of the raw cheese are discarded. The loss incurred amounts to 5–10 % of the weight of raw cheese according to the skill of paring, depth of cutting on a lathe, and the age of the cheese on which the degree of dryness and hence the depth of cutting depends. For cylindrically shaped cheese, the percentage loss of weight (as rind) is roughly 46 times the thickness in inches of the rind pared off. More loss takes place with cracked cheese since the mouldy portions around the cracks have to be cut away and discarded. The reasons for discarding the rind are obvious in that, if included, it would markedly affect the flavour of the product and would not soften with the other parts of the cheese in the cooking process.

In commercial practice the raw cheese is first stripped free from cheese cloth and bandaging. The rinds are then removed either by skilful paring with straight knives or by turning on a special lathe until the "quick" of the cheese is exposed. The latter method is quicker and less wasteful with green cheese or with cheese having moist rinds. With cracked cheese, the dissection of mouldy portions has to be done subsequently with knives. Cracked cheese, however, are not suitable for processing owing to the heavy rind loss, and cheese are rigidly selected for processing. Samples of rind from different manufacturers have shown considerable variation in thickness, dryness and in the amount of processable material cut off with the true rind. The colour, cleanliness and amount of bandage lint clinging to the rind also varies considerably. The rinds can be ground or minced easily into grey powders or mushes.

In the processed cheese industry the yield of rind is considerable and it can be realized at once that the rind possesses considerable value as a feeding stuff for animals when fed appropriately and judiciously. The present paper therefore deals with the composition of a number of representative samples of commercial cheese rinds, which have been collected to examine the nature of the material and its variation in composition and, in some cases, of studying the extent of degradation of the fat and protein in the rind. For this purpose, rinds from ten samples of English, three Canadian and five New Zealand Cheddar cheese were collected and analysed. The rinds from three Stilton cheese were also analysed for comparison. The latter cheese, of course, is not made into processed cheese, but the rind would find its way into hotel refuse possibly used as animal food. In addition, comparison of the composition of the thick mould-infected Stilton rind with the thin bandaged Cheddar rind was considered to be of value.

METHODS OF ANALYSIS

The methods of analyses followed closely those described by Davies *et al.* (1). Ammonia nitrogen was determined by distillation with magnesium oxide at 100° C. The constituents of the ash were determined by the usual routine methods. Fat was determined by ether extraction of the dried powdered rinds by the Soxhlet method and checked by the Gerber method.

RESULTS OF ANALYSIS

The results of the analysis are given in Table I. The values given for the distribution of nitrogen and the inorganic constituents should be compared with those given for ripe cheese by Davies *et al.* (1) and in subsequent publications (2) and Mattick *et al.* (3).

DISCUSSION OF RESULTS

Moisture content. The average moisture content of English Cheddar rind was 16·7, New Zealand 19·9, and Canadian 15·8 %. The range of moisture content of Cheddar cheese is 32–36 %; the moisture content of rinds is thus roughly half that of the cheese. Two rinds from unripe English Cheddar contained 26·2 % of moisture. It is clear that there is a progressive loss of moisture from the rinds of bandaged cheese during the ripening period. The loss is slightly greater in the case of an unbandaged cheese, such as Stilton which also has a rough rind and exposes a greater area for evaporation. Stilton rinds contained an average of 17 % of moisture, and thus the percentage loss is greater than in Cheddar rinds since the moisture content of green Stilton is 37–41 %.

Salt content. On a dry matter basis, Cheddar rinds contained less salt than the inside of the cheese. McDowall & Whelan (4) have studied the diffusion of salt through cheese; they have found a sharp decrease in salt content in the outer rind of cheese as the rind is approached. Evaporation of moisture from cheese rind is a slow process and, although the salt rises in concentration in the water in the outer layers, there is a tendency for salt to diffuse slowly into the inner layers of higher moisture content. This diffusion is not quantitative but approaches it more with cheese with a long ripening period. Thus, if the salt content of a cheese of 33 % moisture content is 1·7 % ($\frac{1}{4}$ oz. per lb.), and if salt content (y) be plotted against moisture content (x), the percentage of salt at any moisture content for quantitative uniformity of salt concentration in cheese moisture is given by the equation $y = 0{\cdot}052x$. All the New Zealand rinds (samples 11–15) are close to but slightly above this line while the English and Canadian Cheddar rinds are well above the line. It appears that the longer storage period and the different texture of the New Zealand cheese have helped the diffusion of salt according to the moisture content of the different zones in the cheese.

Table I. *Composition of cheese rinds*

Sample	English Cheddar 1	2	3	4	5	6	7	7C*	8	8C*	English Cheddar unripe 9	10
Moisture %	16·80	18·31	17·23	16·14	16·93	14·76	21·47	33·36	19·08	35·18	26·16	26·29
% of dry matter:												
Fat	47·36	47·88	46·04	46·03	43·05	47·22	44·65	54·00	50·28	54·39	46·49	44·75
Crude protein	40·76	40·79	42·00	42·25	44·23	41·15	42·83	37·26	40·59	38·03	43·20	43·07
True protein	36·85	36·16	37·63	37·89	38·96	37·42	33·90	29·05	36·60	29·22	41·45	41·51
Ash	4·63	4·78	4·95	4·51	5·93	4·60	6·10	5·40	4·15	5·54	4·96	4·93
Lactose, lactic acid, etc.	7·25	6·55	7·01	7·11	6·79	7·03	6·42	3·34	4·98	2·04	5·35	7·25
Total N	6·336	6·341	6·528	6·567	6·876	6·396	6·658	5·792	6·311	5·961	6·772	6·751
NaCl	1·62	1·76	1·64	1·62	1·84	1·49	1·94	2·81	1·50	2·56	1·64	1·64
Ca	1·302	1·208	1·280	1·233	1·514	1·191	1·535	0·992	1·174	1·147	1·351	1·359
P	0·865	0·769	0·777	0·809	0·855	0·792	0·866	0·773	0·807	0·828	1·001	0·985
Ca/P	1·50	1·57	1·65	1·52	1·77	1·50	1·77	1·28	1·46	1·38	1·35	1·38
% of total N:												
Protein N	90·4	88·7	89·6	89·9	88·3	90·9	79·2	78·0	90·2	76·9	95·9	96·4
Ammonia N	0·72	0·82	0·82	0·94	0·68	0·67	0·42	0·87	0·68	1·66	0·03	0·03
% NaCl in moisture	8·04	7·86	7·89	8·43	9·04	8·60	7·08	5·60	6·34	4·72	4·63	4·60
Acid value of fat	23·1	17·2	31·5	23·7	44·2	20·3	10·2	1·0	9·8	1·0	17·0	14·0

	New Zealand Cheddar 11	12	13	14	15	Canadian Cheddar 16	17	18	Stilton I	II	III	III C*
Moisture %	19·19	17·30	23·21	21·40	18·33	17·94	17·83	11·59	16·11	17·42	18·49	38·50
% of dry matter:												
Fat	51·33	52·40	49·23	49·05	50·97	43·75	44·89	49·44	45·51	44·63	48·94	54·40
Crude protein	39·59	39·70	40·39	40·56	40·42	45·05	45·04	42·76	38·74	38·74	38·80	38·54
True protein	36·37	36·82	35·77	35·57	36·44	42·11	42·00	39·41	30·17	27·20	30·22	24·36
Ash	3·81	3·48	4·34	3·97	3·82	4·36	3·94	4·07	7·22	6·16	4·71	6·02
Lactose, lactic acid, etc.	5·27	4·42	6·04	6·42	4·79	6·84	6·13	3·73	8·53	10·47	7·55	1·04
Total N	6·205	6·223	6·331	6·357	6·337	7·062	7·060	6·702	6·071	6·071	6·081	6·043
NaCl	1·40	1·33	1·85	1·46	1·30	1·46	1·47	1·14	2·88	3·52	2·34	2·43
Ca	1·007	0·984	0·974	1·087	1·102	1·118	1·065	1·308	1·096	1·150	1·398	—
P	0·836	0·816	0·758	0·777	0·781	0·888	0·855	0·775	0·536	0·497	0·638	—
Ca/P	1·21	1·21	1·29	1·40	1·41	1·26	1·25	1·69	2·05	2·32	2·19	—
% of total N:												
True protein N	91·8	93·5	88·6	87·7	90·3	93·2	92·8	92·2	78·5	70·2	78·1	61·8
Ammonia N	0·53	0·59	1·07	1·28	1·03	0·50	0·39	1·06	5·53	8·49	8·30	11·30
% NaCl in moisture	5·89	6·36	6·11	5·37	5·78	6·69	6·79	11·40	15·02	16·70	10·33	2·46
Acid value of fat	30·8	36·2	84·0	33·1	70·9	44·1	19·0	22·0	49·3	55·4	43·3	20·4

* The letter C after the sample number denotes the cheese corresponding to the rind.

Protein and soluble nitrogen compounds

The dry matter of rinds contains a higher total nitrogen content than the inside of the cheese. Part of the increase is due to loss of salt and fat from the rind. In two samples where the rinds were compared with the inside of the cheese (nos. 7 and 8) the increases were 5 and 16 %. If, however, the total nitrogen is calculated for the non-fatty matter, the values are lower for the rinds than for the inside of the cheese by about 2 %. The explanation of this is that during protein degradation in ripening, soluble nitrogen compounds, which on the whole contain a higher percentage of nitrogen than the original curd, diffuse from the rind to zones of higher moisture content, thus relatively impoverishing the rind in nitrogen. Some loss as ammonium salts or as volatile ammonia from the action of moulds also accounts for this low value.

The degree of protein breakdown in the rind is invariably much less than in the interior of the cheese. This may be due (*a*) to a lower moisture content slowing down the rate of degradation, since moisture content is known to affect proteolysis in cheese, and (*b*) to a slow diffusion of soluble nitrogenous products to zones of higher moisture content in the interior of the cheese. The ammonia content is also less in the rind. This is the case even in Stilton cheese where mould growth is expected to form a considerable amount of ammonia.

Fat. On a dry-matter basis, the fat content of rinds is lower than that of the interior of cheese by 8–12 %, so that it is obvious that there is considerable loss. The loss is partly accounted for by diffusion from the dry rind into the bandage and, to a small extent, through breakdown and loss of volatile acids. Fat could only diffuse into the interior of cheese if there were a continuous fat phase and since this is not present in cheese, loss from this cause is not to be expected. A film of fat forms rapidly on the outside during the desiccation of a freshly cut surface of cheese. The capacity of the bandage to absorb fat is high. Two of such bandages from ripe cheese contained 41 and 42 % of fat of acid value 92·8 and 95·3 respectively. That is, the bandages are capable of absorbing over two-thirds of their weights of fat from cheese rind. Some bandages are greased with butterfat. In the present work, it is not known whether the bandages of the cheese from which the rinds were taken were greased or not. In any case none of the bandage fat contributed to the fat in the rinds.

Condition of the fat. Rind fats in all samples showed a relatively high content of free fatty acids, which was higher than that for cheese fat but much lower than that of the fat in the bandage. If the free fatty acids are calculated as oleic acid, bandage fats were found to contain 48 %, rind fats 16 % and the fat from the interior of cheese 0·5 %. The action of moulds is undoubtedly the major cause of this high degree of fat breakdown. The degree of breakdown of rind fat is variable and cannot be correlated, for instance, with the moisture content. The fat, when separated and tasted, did not possess a tallowy flavour

and could be regarded almost as wholesome except for a slight ketonic rancidity. The characteristic taste of cheese rind was present in the rind fat.

Mineral ingredients. Both calcium and phosphorus contents were higher in the dry matter of rinds than in that of the interior of the cheese. Calcium increased to a greater extent than phosphorus, with the result that the Ca/P ratio increased to 1·6 as against a value of 1·4 for cheese. It is natural to assume that the *p*H of rinds will vary with that of the interior of the cheese (*p*H 5·2–5·8) and that a considerable amount of Ca and PO_4 will be in ionic form. Probably the phosphate ion diffuses into zones of higher moisture content at a more rapid rate than Ca, and Ca is also held back in the rind layer partly as calcium soap. The rinds are a comparatively rich source of mineral matter.

Fibre. Some fibre determinations were done on 5 g. samples of rinds. The amount in clean rinds was negligible (less than 0·2 %) and consisted of cotton lint from the bandages and some mould spore cases and broken mycelia. The fibre content is not of serious consequence and the quality of a sample of rind can easily be assessed from a cursory examination or the presence of portions of bandage.

Summary

Rinds from home-produced and Dominion Cheddar cheese contain from 15 to 20 % of moisture, but those from unripe cheese contain up to 27 %. Salt tends to diffuse into the interior of cheese while the rind is slowly drying, but the rate of diffusion is not quantitatively proportional to the moisture content, although closely approached in cheese requiring a long period of ripening.

There is an appreciable loss of fat from the rind through absorption by the bandage. The fat has a high acid value and carries the characteristic taste of the rind.

Rinds have a higher crude protein content than the interior of cheese; the extent of protein breakdown is less but can be correlated with that in the interior of the cheese.

Rinds contain higher calcium and phosphorus contents in the dry matter and a higher Ca/P ratio than the interior of cheese.

The fibre content of rinds is negligible. The content can be gauged from a visual inspection of a sample for portions of bandage.

The author is indebted to Mr Capstick (Aplin and Barrett's, Yeovil), to Mr Roberts (Kraft Cheese Co.) and to Mr Freeth (J. Nathan and Co.) for the samples of rinds from commercial cheese.

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637.321:539.2

203. AN APPARATUS FOR MEASURING THE ELASTIC AND PLASTIC PROPERTIES OF CHEESE CURD

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(With 1 Figure)

In judging the consistency of cheese curd by hand testing, two distinct properties are generally assessed: first the firmness is estimated by pressing the back of the hand on to the curd surface, and secondly, the type of break is judged by causing a crack to form in the curd by means of the finger and thumb.

The advisability of developing an objective test to measure curd firmness and strength has long been realized, but hitherto most of the methods proposed have been concerned only with the second of these properties, nor have the tests been able to measure in any quantitative sense the *type* of rupture, which is of interest to the cheesemaker, but have concentrated on determining the *force* required to cut the curd (e.g. Alleman & Schmid (1), Hill (2), Lauder *et al.* (3), Burgwald & Armstrong (4), etc.).

In designing a method for measuring the firmness of uncut curd, it is clearly advisable to distinguish those physical properties of the curd which can be defined in absolute (c.g.s.) units, so that the criteria finally used may be reproducible, and may be as independent as possible of the shape and size of the apparatus itself. Preliminary experiments showed that normal curds for several types of cheese just prior to cutting, when given quite big compressions for periods of about $\frac{1}{2}$ min., will recover elastically a very high proportion of these deformations. Although the elastic reaction is not instantaneous (elastic after-effect is present), this means that the elastic moduli of the curd will be by far the most important criteria of its firmness.

It is safe to assume that the bulk-modulus of curd is very large, and that Young's modulus will therefore be a simple multiple of the shear modulus, so that only one elastic modulus need concern us. If the area under compression is small compared with the size of the vat, the shear modulus will be proportional to the ratio of the force used to compress the curd to the elastic deformation produced.[1]

An apparatus, shown in Fig. 1, was made to observe these deformations. A light metal tray (A) diameter 15 cm., with a flanged edge height 0·5 cm.,

[1] I am indebted to Dr R. Eisenschitz for drawing my attention to the following formula:

$$\epsilon = F/8rn,$$

where ϵ is elastic compression, F is force of compression, r is radius of cylindrical tray on which load is applied, n is shear modulus.

is carefully placed on the curd. To avoid any danger of contaminating the curd with metal the part of the tray which comes into contact with the curd is painted with a resistant paint. The apparatus is clamped to a suitable frame so that it can be lowered into a vertical position just above the tray. The

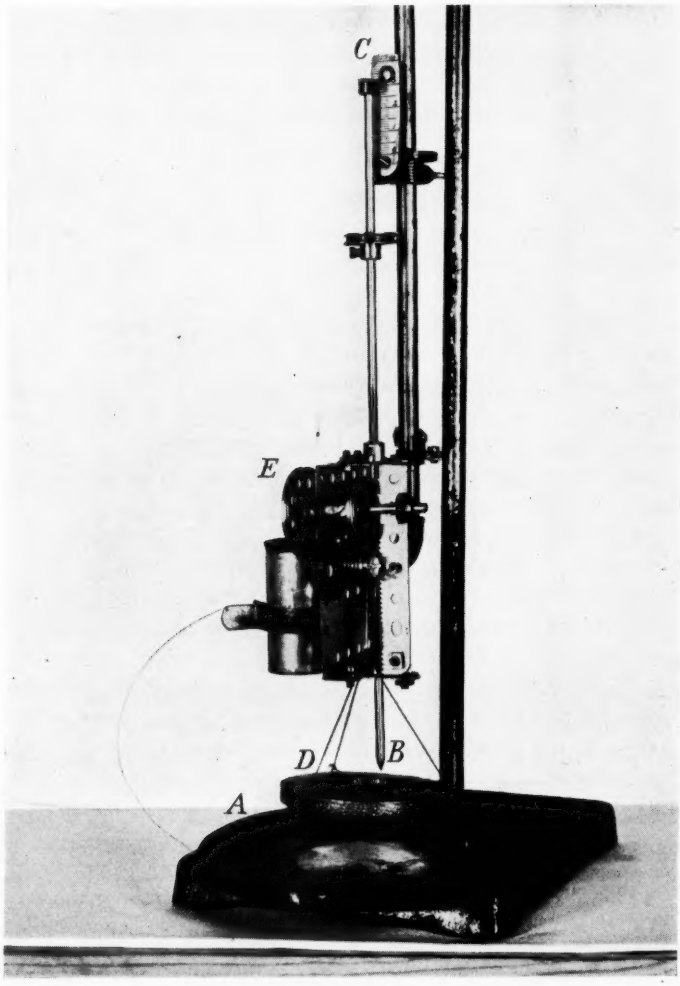

Fig. 1.

steel (Meccano) rod (*B*), pointed at the lower end, can be raised or lowered by means of a worm, and its changes in height can be read on the scale (*C*). A flat disk at the top of the rod makes it possible to avoid parallax. The tray (*A*) is connected by a very light wire to a torch bulb and battery attached to the apparatus, to which the other terminal of the battery is earthed, so that the

bulb is lighted as soon as the point of the rod touches the centre of the tray. An annular lead weight of 300 g. (D) (which is found to be the greatest load which curds rather softer than normal will stand without rupture) can be lowered on to the tray by means of the wheel (E). The test is performed as follows: The rod (B) is screwed down till it just makes contact with the tray, and the reading on the scale (C) is recorded (C_1). The weight (D) is gently but quickly lowered on to the tray and a stop-watch is started. After 25 sec. the rod (B) is screwed down again until contact is remade, and at 30 sec. a reading on the scale (C_2) is taken, the weight being at once raised, and the rod screwed up so that the tray is free to rise as the curd recovers. This releasing process may take a few seconds, but since a period of a further 30 sec. is allowed to elapse before the recovered position (C_3) is read, the slight delay involved is unimportant. The test is generally repeated immediately without changing the position of the tray, and although there is some slight tendency to elastic fatigue, agreement between the two loadings should be very close. The scale is read to tenth parts of a millimetre, and readings should be reproducible to about twice this distance, though in some cases larger errors occur. A further test can be done if the duplication is not satisfactory. Since the loads and times are always the same, the elastic deformation, $\epsilon = (C_3 - C_2)$ is inversely proportional to the shear modulus, and the non-recovered deformation, $\pi = C_1 - C_3$, inversely to the viscosity.[1]

An investigation is now being undertaken to study the variations in physical properties of curd in relation to the opinions expressed by experienced cheese makers, and a large number of data is being collected using the above technique. The results of this investigation will be published in due course. Data from a run in an average normal Cheddar curd when ready for cutting are given in Table I.[2]

Table I

1st compression	C_1 1·71 C_2 0·48 C_3 1·64	$\epsilon_1 = 1{\cdot}16$ $\pi_1 = 0{\cdot}07$
2nd compression	C_1 1·64 C_2 0·41 C_3 1·59	$\epsilon_2 = 1{\cdot}18$ $\pi_2 = 0{\cdot}05$

These results should only be taken as typical for the particular dairy in which they were obtained. It will be seen that the elastic deformation is much greater than the plastic, and that the percentage error in π is correspondingly high. There is a general tendency for the second ϵ to be higher than the first, suggesting slight elastic fatigue, and for π_2 to be lower than π_1,

[1] Since the viscosity of a material such as curd is not independent of strain, the viscosity here discussed must be taken as a mean value.

[2] I am indebted to Miss V. Coppen, who is undertaking these measurements, for the figures given in the table.

presumably due to a kind of work-hardening. A small amount of whey is exuded during the loading, but does not interfere significantly with the test.

Although the nature of the break of the curd, and possibly even the tearing strength, which depend on properties other than shear modulus, may have some significance in defining the condition suitable for cutting, it seems probable that the shear modulus, which is inversely proportional to ϵ, is by far the most important single factor, and it is suggested that its measurement will prove useful in standardizing cutting conditions. The effect of size and shape of vat on ϵ for a curd of given shear modulus has not yet been investigated in detail either from the practical or theoretical point of view, but it is found that a considerable difference in size of vat does not alter ϵ greatly. The question of applying corrections for vats of different dimensions is being considered. Direct measurements of Young's modulus on cylinders of curd have been tried, but unless the curd is firmer than is usual for cutting, the cylinders will not stand up under the action of gravity during the performing of the test even when very small test pieces are used.

Summary

An apparatus is described for measuring the elastic and plastic deformations of cheese curd under a compressive load at the time when it is ready for cutting. A figure inversely proportional to shear modulus can be obtained with considerable accuracy, and is believed to serve as the best single criterion of "firmness" in deciding the optimum time to cut the curd.

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637.127.6:637.128

204. THE METHYLENE BLUE REDUCTION TEST AND THE KEEPING QUALITY OF MILK

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The keeping quality of milk is a factor which is of considerable importance from the commercial aspect, yet the relationship which exists between the keeping quality and the bacteriological standards as laid down in the Milk Special Designations Order, 1936[1], has not been fully explored. Wilson (1) compared the keeping quality at 17·5° C. of samples of mixed morning and evening milk with the reduction times at different temperatures and found, in each case, a relatively poor correlation. Since the samples consisted of mixed morning and evening milk it is not possible to state the age of the milk when the tests were commenced. Much of the Tuberculin Tested milk sold in the Provinces is bottled on the farm, and consequently the milk produced in the morning is bottled separately from that produced in the evening. Under the 1936 Order samples of bottled Tuberculin Tested milk should be subjected to the reduction test (at 37° C.) within 2 hr. of the time when the sample was taken. In many cases, therefore, the morning milk is less than 6 hr. old when the test is commenced. By the end of the keeping quality period the numbers of the different species of organisms will have undergone considerable change and will probably bear little relation to the numbers present a few hours after production of the milk. Moreover, the rate of growth of the different species during the reduction test at 37° C. will be distinctly different from their rates of growth at 15° C. during the keeping quality test. It is not to be expected, therefore, that there will be a good correlation between the results of the reduction test and the keeping quality. It was decided, however, to investigate the extent of the correlation and to determine if it could be improved by holding the milk for a longer period than 2 hr. at a standard temperature of 15° C. before submitting the samples to the reduction test at 37° C. Samples of morning milk were therefore maintained for 1, 7, 16 and 23 hr. at 15° C. before being tested, and the results compared with the keeping quality of the milk when held at a temperature of 15° C. It was not possible to make an accurate estimate of the keeping quality, as there is no satisfactory method for determining the end-point of the test. Differences in acuity of personal senses and the fact that unpalatability in milk is not due to any one factor

[1] Under this Order, Tuberculin Tested and Accredited milk "when tested in accordance with a prescribed method must not decolorize methylene blue within 4½ hours if the sample is taken at any time from 1st May to 31st October; or within 5½ hours if the sample is taken at any time from the 1st November to the 30th April" (Memo. 139/Foods, Ministry of Health—Bacteriological Tests for Graded Milk).

but a combination of many, must inevitably lead to a comparatively large error. However, the difficulties were, to some extent, overcome by using a suggestion made by Hiscox *et al.*(2) of adding methylene blue to a portion of the sample and maintaining it at 15° C. The end-point of the keeping quality period approximates to the point when the colour of the dye is discharged. In every case this test was supplemented by tasting the milk and testing it for clotting on boiling. In this experiment the end-point of the keeping quality of the milk was considered to be reached when the milk had an unpleasant flavour and was therefore unsuitable as a beverage. In every case the colour of the methylene blue was discharged, but the milk did not necessarily clot on boiling. The observations were made, so far as was practical, at 5 hr. periods. Thus a fairly reliable, although somewhat wide, classification of the milks as regards keeping quality could be made.

Each of the 200 samples of milk examined were produced about 7 a.m. and cooled at the farm. Samples were taken at 10 a.m. and despatched immediately to the laboratory. Each sample was divided into six portions and treated as follows:

Portion	Temperature and time at which the sample was maintained before the reduction test was commenced
No. 1	1 hr. at room temperature
No. 2	7 hr. at 15° C. and then 16 hr. at 0–4° C.
No. 3	7 hr. at 0–4° C. and then 16 hr. at 15° C.
No. 4	23 hr. at 15° C.

Two portions, nos. 5 and 6, were maintained at 15° C. and used for the keeping quality tests.

Thus portion no. 1 was held for 1 hr., portion no. 2 was held for 7 hr., portion no. 3 for 16 hr. and portion no. 4 for 23 hr. at 15° C. before the reduction tests were commenced.

The results of the reduction tests and the keeping qualities have been classified and are given in Tables I–IV.

It is clear from Table I (samples examined after 1 hr. incubation at 15° C.) that there is a poor relationship between the reduction time and the keeping quality for milks which remained sweet for less than 34 hr. The reduction times ranged from 2½ to 8 hr. or more. Forty-nine of the samples which kept sweet for less than 34 hr. failed to reduce the dye within 5½ hr. and sixty-five samples to reduce the dye within 4½ hr. That is to say, 53·2 % of these samples satisfied the "winter" standard and 70·6 % the "summer" standard as laid down in the Milk Special Designations Order. This table also shows that, although samples of milk which have a good keeping quality generally tend to reduce methylene blue slowly, it cannot be assumed that this is so in every case, for twelve samples out of thirty-seven which kept sweet for less than 28 hr. took 7 hr. or longer to decolorize the dye.

Table II (samples examined after 7 hr. incubation at 15° C.) also demonstrates the wide variation which may occur in the reduction time of milk which

kept sweet for less than 34 hr. In the case of those samples which kept sweet for 29–33 hr. the reduction time ranged from ½ to 8 hr. On the other hand, those milks which kept sweet for less than 29 hr. all reduced the dye within 5½ hr. It is also interesting to note that five samples which had a relatively good keeping quality (44–48 hr.) failed the "winter" standard of 5½ hr.

Table I. *Samples examined after* 1 *hr. incubation at* 15° C.

Reduction time (hr.) at 37° C.	No. of samples and keeping quality at 15° C. in hours						
	24–28	29–33	34–38	39–43	44–48	49–53	54–58
½	.	.	.	.	.	.	.
1	.	.	.	.	.	.	.
1½	.	.	.	.	.	.	.
2	.	.	.	.	.	.	.
2½	3	.	.	.	.	.	.
3	2	3	.	.	.	.	.
3½	2	1	.	.	.	.	.
4	3	4	.	.	.	.	.
4½	3	6	2	.	.	.	.
5	6	5	.	.	.	.	.
5½	1	4	1	.	.	.	.
6	2	4	1	.	.	.	.
6½	3	5	1	.	.	.	.
7	5	3	6	1	1	2	1
7½	.	1	.	1	1	.	.
8	7	19	12	8	25	18	27
Total no. of samples	37	55	23	10	27	20	28

Table II. *Samples examined after* 7 *hr. incubation at* 15° C.

Reduction time (hr.) at 37° C.	No. of samples and keeping quality at 15° C. in hours						
	24–28	29–33	34–38	39–43	44–48	49–53	54–58
½	2	1	.	.	.	.	.
1	4	2	.	.	.	.	.
1½	6	5	1	.	.	.	.
2	2	6	.	.	1	.	.
2½	4	4	.	.	.	.	.
3	3	9	1	.	.	.	.
3½	1	3	.	.	.	.	.
4	.	.	.	.	3	.	.
4½	6	8	5	.	.	.	.
5	1	2	2	.	.	1	.
5½	4	6	2	1	1	2	1
6	.	3	4	3	1	3	.
6½	.	3	4	1	3	1	1
7	.	5	1	3	9	6	3
7½	.	2	.	1	5	3	8
8	.	3	2	1	4	2	15
Total no. of samples	33	62	22	10	27	18	28

In Table III (samples examined after 16 hr. incubation at 15° C.) the results of only 75 of the 200 samples are recorded, but it will be seen from this table that there is a better correlation between the reduction time and the keeping quality. Although the range of the reduction times for milks which kept sweet for 29–33 hr. is wide, all the samples reduced the methylene blue within 4½ hr., i.e. they failed to pass both the "summer" and the "winter" standards.

In Table IV (samples examined after 23 hr. incubation at 15° C.) there is a relatively good agreement between the keeping quality and the reduction time. The ranges of the reduction times are much smaller, except in those cases where the milk remained sweet for over 48 hr. The increased spread of

Table III. *Samples examined after* 16 *hr. incubation at* 15° C.

Reduction time (hr.) at 37° C.	No. of samples and keeping quality at 15° C. in hours						
	24–28	29–33	34–38	39–43	44–48	49–53	54–58
½	.	.	.	.	.	.	.
1	6	6	.	.	.	.	.
1½	4	9	2	.	.	.	.
2	.	3	.	.	.	.	.
2½	.	2	3	.	.	.	.
3	.	5	1	2	.	.	.
3½	.	1	.	1	.	.	.
4	.	.	.	.	.	.	.
4½	.	1	.	1	3	1	.
5	.	.	.	.	3	.	1
5½	.	.	.	.	.	2	.
6	.	.	1	.	.	3	2
6½	.	.	1	.	3	.	4
7	.	.	.	.	1	.	3
7½	.	.	.	.	.	.	.
8	.	.	.	.	.	.	.
Total no. of samples	10	27	8	4	10	6	10

Table IV. *Samples examined after* 23 *hr. incubation at* 15° C.

Reduction time (hr.) at 37° C.	No. of samples and keeping quality at 15° C. in hours						
	24–28	29–33	34–38	39–43	44–48	49–53	54–58
½	29	26	1	.	.	.	.
1	3	23	2	.	.	.	.
1½	1	13	13	.	.	.	.
2	.	.	5	5	1	.	.
2½	.	.	1	4	4	.	.
3	.	.	.	1	14	.	.
3½	.	.	.	.	6	.	.
4	.	.	.	.	.	6	.
4½	.	.	.	.	2	8	3
5	.	.	.	.	.	4	3
5½	.	.	.	.	.	.	5
6	.	.	.	.	.	.	8
6½	.	.	.	.	.	.	6
7	.	.	.	.	.	.	3
7½	.	.	.	.	.	.	.
8	.	.	.	.	.	.	.
Total no. of samples	33	62	22	10	27	18	28

the range of reduction times with milks which have a keeping quality of more than 50 hr. is of particular interest and demonstrates that there is a change in the relative numbers of organisms of different species during the keeping quality period. This change cannot be forecast in these milks by the reduction test.

The correlation coefficients for the reduction time and the keeping quality

for the different age periods at which the reduction test was started are as follows:

Age of the milk when tested hr.	Correlation between the reduction time and the keeping quality
23	0·954 ±0·0043
*16	0·893 ±0·015
7	0·660 ±0·055
1	0·442 ±0·059

* Only 75 samples examined.

In the calculation of the correlation coefficients only those samples were considered which had a reduction time of 7½ hr. or less, as no record was made of the actual reduction times in excess of 7½ hr.

The correlation coefficients fully confirm the conclusions which are indicated from a general survey of the four tables (Tables I–IV), namely, that the agreement between the reduction time and the keeping quality improves as the period of incubation of the sample at 15° C. is extended. The explanation of this appears to be that there is during the keeping quality period a progressive change in the numbers of the different species of organisms, and that the type which is predominant at any particular time varies and is not necessarily the predominant type when the milk becomes unpalatable. Further, the type of organism which predominates during the reduction test is often different from the type which predominates at any particular time during the keeping quality period, except in those cases where the start of the reduction test approaches the end of the keeping quality period. This also explains why the range of the reduction time, when the milk is incubated for 26 hr., is narrower with milks which have a relatively short keeping quality, and is wider with those which have a relatively long keeping quality.

From these experiments it may be concluded therefore, that by incubating the milk for a period of 15–24 hr. at 15° C. a much better correlation can be obtained between the reduction time (at 37° C.) and the keeping quality, than if the milk is examined within a few hours of its production. In fact, it may be said that examining the milk within 4 hr. of the time of its production gives a very misleading indication of its keeping quality and hence a false sense of security from the commercial aspect.

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BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION E. THE DISEASES OF DAIRY CATTLE

I. Mastitis
II. Contagious abortion
III. Tuberculosis

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I. MASTITIS

Bacteriology

Perhaps the two most significant results of extensive research into bovine mastitis during the past few years are first, the recognition of a fairly well-defined species of streptococcus as the principal cause of chronic bovine mastitis, and secondly, general agreement in the biochemical and serological characteristics of the causative agent. The classification of the streptococci has been reviewed recently by Sherman (1). The attempted division of the streptococci into species is based primarily on the Lancefield serological method of grouping supplemented by cultural and biochemical tests. Eight main serological groups are distinguished, and Group B includes mastitis streptococci to which the name *Str. agalactiae* is commonly assigned.

Within this group Stableforth (2) formerly recognized three serological types (*a*, *b*, *c*), but as a result of examining some 2000 further strains (3) isolated in Great Britain, he has redesignated these original types as 1*a*, 2*a*, 3*a*, and two further subtypes 1*b* and 3*b* have been distinguished. These strains were examined by direct agglutination, agglutination-absorption and precipitation tests. The characters of thirty-three strains of *Str. agalactiae* isolated in other European countries conformed in the main to the British subtypes 1*a* and 2*a*. Stableforth (4) has also surveyed the incidence of these various serological types in fifty-two dairy herds. Streptococci were isolated from 1546 quarters of 782 cows. In nearly all cows having one or more quarters of the udder infected, the characters of the streptococcus conformed to one subtype. About 10 % of these pure infections were due to type 2, the remainder being equally distributed between type 1 (usually 1*a*) and type 3 (usually 3*a*). In thirty-eight herds out of a total of forty-five, 78 % of the individual infections were alike and in fifteen herds all were alike. Of these thirty-eight herds, twenty-three were infected with type 1, three with type 2 and twelve with type 3. In view of the tendency towards type specificity of infection within herds Stableforth considers that this lends support to the view that mastitis due to *Str. agalactiae* is a purely contagious disease.

Plastridge & Hartsell (5) have examined 157 strains of weakly haemolytic streptococci recovered from fresh milk samples. Of these 122 possessed the biochemical characters of *Str. agalactiae*, and precipitin tests showed that they belonged to Lancefield's Group B. Most of these strains were isolated from animals with a history of permanent infection, but thirty strains, although biochemically resembling *Str. agalactiae*, differed from this organism serologically and by producing infection of relatively short duration, with or without evidence of mastitis. These cultures were tentatively designated as *Str. pseudo-agalactiae*.

The biochemical characters of this group have been investigated by Hansen (6) and by Engelbrecht (7). The behaviour of the strains examined by them was found to agree with that accepted to be characteristic of *Str. agalactiae*.

Experimental infection. Although mastitis has been induced fairly regularly under experimental conditions by injecting culture directly into the udder through the teat, it must be said that the doses used have probably contained far more organisms than would gain access to the teat under natural conditions. For this reason the experiments of Little (8) are of interest because they serve to demonstrate the effect produced by minimal doses.

Little describes a series of experiments designed to infect the healthy quarters of lactating heifers by injecting graded doses of *Str. agalactiae* culture into the teat duct. It was found that when a small amount of pure culture or of a 10^{-1} dilution was introduced into the canal, mastitis developed in twelve quarters of five heifers after a single inoculation. A 10^{-2} dilution (approximately 6000 organisms) caused mastitis in six quarters after one inoculation and in three quarters after two; in one quarter the disease was established only after four inoculations. With the smallest inoculum (containing about 500 streptococci) thirty-seven inoculations were required to induce infection in seven out of nine quarters, while two remained uninfected after eight and nine inoculations respectively. In a further series of experiments Little (9) tried to establish infection by applying streptococcus culture to the teat meatus either by rubbing or by natural suction. It was found difficult to set up the disease in heifers by these methods although older cows responded more readily. The resistance of heifers was thought to be related to the intact duct and sphincter of the teats.

Diagnosis

Direct cultural methods. A selective medium for the cultivation of *Str. agalactiae* has been described by Pagnini (10), and its constitution is based on the media previously described by Klimmer *et al.* (1928) and Edwards (1933). The medium contains the following ingredients: 90 ml. agar, containing 1 g. mannite and arabinose per 100 ml., 10 ml. horse serum, 0·2 ml. of a 1 % aqueous solution crystal violet, and 0·5 ml. of a 1 % alcoholic solution of brom-cresol-purple. Colonies of *Str. agalactiae* do not change the colour of this medium, whereas those of *Str. lactis* and other streptococci produce yellow zones. Streptococci may be further differentiated by growing them in broth to which various dyes have been added, e.g. Loeffler's blue, gentian violet, etc. *Str. lactis* grows profusely in broth containing these dyes and causes decolorization especially in Loeffler's blue, whereas *Str. agalactiae* does not grow well and does not produce any change in colour. Hartmann (11) found that a broth medium containing 0·05 % sodium azide inhibited saprophytic organisms in milk and at the same time did not interfere with the growth of mastitis streptococci. Janonis (12) tried the inhibitory action of thirty substances on strains of mastitis streptococci, micrococci, *Bact. coli*, *Str. lactis* and *Br. abortus*. The most pronounced effect was produced by salts of telluric acid which inhibited strains of *Bact. coli* and micrococci in concentrations which did not interfere with the growth of mastitis streptococci. Klimmer & Weiske (13) found that cultural recognition of mastitis streptococci in milk samples containing other bacteria was not simplified by previously heating the samples at 55–65° C. for 5–30 min., nor was there any advantage in incubating samples at 40–42° C. previous to cultivation.

Hotis (14) described a test for the recognition of *Str. agalactiae* in milk samples which consisted of the addition of 0·5 ml. of a sterile 0·5 % aqueous solution of brom-cresol-purple to 9·5 ml. milk carefully collected from the cow. After mixing, the sample was incubated for 24 hr. at 37° C. and the results observed. A charac-

teristic change in the colour of the sample after incubation, together with the occurrence of flakes or balls of growth, was taken to denote the presence of *Str. agalactiae*. The examination of 753 samples by the Hotis test and by the blood agar plate method showed that 668 samples were positive to both tests and forty-seven negative, so that there was agreement in 95 % of the samples. Hotis states that in order to obtain clear cut results it is necessary to collect the milk as aseptically as possible. This test has not given such good results in the hands of Bryan & Devereux (15). They examined composite milk samples from 310 cows by the microscopic test of incubated samples (which was used as the standard for comparison), the blood agar plate and the Hotis tests. The plating method detected forty-six (or 86 %) of fifty-three samples positive to the microscopic test, while the Hotis test was positive with 52 % after 24 hr. incubation and with 62 % after 48 hr.; 36 % of the samples gave doubtful readings to the Hotis test and the authors consider that this was a serious defect to this test. Inconsistencies were again obtained in the results of the examination of samples negative to the microscopic and blood agar tests.

The value of a test dependent on the recognition of streptococci in incubated milk samples has been questioned by Plastridge *et al.* (16). For the purpose of selecting the most efficient method of detecting "shedders" of mastitis streptococci in freshly drawn milk samples, six different methods were compared. Samples from 360 quarters were examined and seventy-nine (21 %) showed laboratory evidence of mastitis. The following percentage positive results were given by the various tests employed:

(1) direct microscopic examination of films (whole milk), 3·8;
(2) direct microscopic examination of films of sediment, 8·9;
(3) blood agar plates streaked with a loopful of whole milk, 60·8;
(4) blood agar plates inoculated by standard dilution, 76·0;
(5) blood agar plates streaked with a loopful of sediment from 10 ml. milk, 77·2;
(6) microscopic examination of films from samples incubated overnight, 98.

These workers state that in the absence of other laboratory evidence of mastitis, the finding of streptococci in incubated milk samples should not be taken as conclusive evidence of infection with the organism which is commonly responsible for chronic bovine mastitis. The significance of the finding of streptococci in such instances can be determined only by isolation and identification of the streptococcus found in the sample.

With a view to estimating the degree of mastitis in the mixed milk of a herd Pullinger (17) attempted to fix a standard based on the *Str. agalactiae* count. Owing to the marked variation in the weekly count of *β haemolytic streptococci*, it was found impossible to set up a standard count above which a producer might be penalized. An estimate of the infection present is only to be obtained by four or five cultural examinations at weekly intervals. Microscopic examination of gravity cream or of centrifuged deposit for the presence of streptococci or leucocytes can be recommended as auxiliary tests.

Indirect methods. Bryan & Trout (18) examined by indirect methods milk samples from three herds two of which were infected with mastitis. In general, they found that salty flavour, increased chloride content, leucocyte count and bacterial count are shown by mastitis milk, but point out that other factors such as stage of lactation, etc., may produce similar changes. The methylene blue rating of infected composite samples was lower than that of non-infected samples and the same was also true of quarter samples. None of these determinations in itself, however, could be taken as a criterion of streptococcus infection. The method of chloride determination used by Rosell and his claim that this test alone can be used for the detection of mastitis

have been severely criticized by Blood & Rowlands (19). These authors found that Rosell's method gave high results and was, further, unsatisfactory in use. Even when reliable methods of chloride determination were employed many infected quarter samples of milk showed chloride contents within the normal limits of variation (0·07–0·12 %), and many cows whose milk had chloride content well above these limits were free from all evidence of disease by cultural tests. A series of milk samples taken at consecutive milkings were analysed by Johns & Hastings (20) to determine the reliability of indirect biochemical tests (chlorides, catalase, *p*H) for the detection of chronic mastitis. It was found that infected quarters not infrequently gave normal milk while many non-infected quarters yielded milk giving definitely abnormal reactions. Furthermore, the reactions to these tests frequently fluctuated widely from milking to milking from both infected and non-infected quarters. Of the three tests used, the catalase test was found to be the most sensitive indicator of infection.

Hopkirk (21) has investigated the cell content of milk as a means of diagnosing mastitis. Loops of cream of the first milk after standing for a certain time were smeared on slides, defatted, stained and examined for leucocytes. Results were shown to be correlatable with plate counts and with the types of organisms present. Assessment figures 0–6 are given according to the average number of leucocytes per field. It is claimed that heifers with normal udders have practically no leucocytes in gravity cream.

Anderson *et al.* (22) found that mastitis decreases the curd tension of milk as measured by the Hill curd test, streptococci groups A and B and staphylococci being equally effective in this respect. In mastitis-free milk high correlation coefficients were found between casein content and curd tension and between CaO and P_2O_5 and curd tension. In milk from infected quarters these correlations were markedly reduced. It is interesting to note that Rowland (23) also found a correlation between the presence of mastitis and a decreased casein content in the milk of cows showing low solids-not-fat. Hadley (24) investigated the different rates of coagulation of normal milks and infected milks after the addition of rennet. 0·1 ml. of a solution of rennet diluted with fifty parts distilled water was added to 5 ml. of milk and allowed to stand for 1 hr. at room temperature (72–82° F.). Samples coagulating within this period were regarded as normal, while a longer period was taken as evidence of mastitis infection. Samples of quarter milk were examined by a combination of bacteriological and other methods and the results compared with those of the rennet test. Of 314 quarter samples, 61 (21 %) were found to be abnormal and 247 (79 %) normal to the combination of tests, while the corresponding figures for the rennet test were 20 and 80 % respectively. No details are presented for the bacteriological methods employed except that the diagnosis was based on the streptococcus colony count.

The effect of chemical changes in the milk of individual cows or in the bulk milk of a herd due to mastitis has been studied by Dahlberg *et al.* (25). They point out that "most chemical and bacteriological analyses of milk in mastitis studies have dealt with fore-milk and in some instances with strippings or the complete milking from individual quarters. In commercial practice cows are completely milked and the milk from all quarters is mixed together, while milk of abnormal macroscopic appearance is rejected as soon as detected." Only cows whose udders were free from active inflammation were used for this study and they were divided into three groups according to the reaction to certain tests applied to the milk. The total day's milk of each cow was mixed with that of the other cows in the same group and a composite sample submitted weekly to a detailed chemical analysis. It was found that the presence of latent mastitis caused very slight differences in the chemical composition of the mixed milk of these groups, so long as the milk remained normal in appearance.

These slight differences included a decrease in lactose, specific gravity, skim milk solids and curd tension while the chlorides and albumins were slightly increased. These changes, however, were not greater than variations in the chemical composition of milk between two herds of the same breed.

Control

By preventive methods. Successful results in the control of mastitis in seven herds have been recorded by Plastridge *et al.*(26). The diagnosis was made by the evidence of a leucocyte count and the presence of *Str. agalactiae*. After testing, the animals in the herds were segregated into negative, suspicious and positive groups and milking was performed in this order. In one herd the initial incidence of infection was 33 %, and 22 % new infections occurred during 18 months before complete segregation. After segregation the total incidence was reduced to 16·6 % and the new infections fell to 3·3 % in the next year and to zero in the following year. In the remaining herds similar results were obtained. The authors state that "three important factors must be considered in the control of contagious mastitis, (*a*) that grouping the positive animals at one end of the byre and milking them in the order suggested greatly reduces but does not entirely prevent spread of infection, (*b*) that apparently infection may be transmitted to negative animals by contact with floors that have been infected by carrier animals, (*c*) that isolation of the positive animals, rather than placing them at the end of the milking string is necessary to prevent completely the spread of infection of streptococcal mastitis".

Hucker & Harrison(27) describe the results of control work in three herds over a period of three years; weekly quarter samples were examined culturally and biochemically. One of the herds (Herd E) was free from infected animals at the beginning of the experiment, in the second (Herd W) six out of seventeen were infected and the third (Herd N) fifteen out of thirty-five were infected. New infected animals from the first herd were removed from time to time to the other two herds. Strict precautions were taken in the first two herds to minimize the spread of infection by milking reactors last and by dipping the teat cups and washing the hands in hypochlorite solution between milkings; in the third herd the milking order was not strictly maintained. During the three-year period it was found impossible to maintain healthy cows free from mastitis streptococci in the milk and 22 % of the clean cows in Herd E developed infection, 26 % in Herd W and 64 % in Herd N. From these results the authors state that "there is little significance to the procedure of milking infected cows last from the standpoint of reducing the incidence of mastitis streptococci in the milk or the possibility of reducing the spread of infection". Throughout this work it is not clearly stated that efforts were being made to control infection due to *Str. agalactiae* which, so far, is the only form of mastitis reported to be controllable.

The successful control of streptococcus mastitis must primarily depend on an exact knowledge of the habitat of the causal organism. It would appear from work carried out by Hucker(28) that there must be factors concerned with the mode of infection other than transmission from infected to healthy cows during milking. Hucker studied the habitat of mastitis streptococci in the apparently normal udder tissues of cows and of virgin heifers and calves. Cultural examination was made of tissue aseptically removed from the udders of forty-six such animals which, previous to slaughter had shown no clinical evidence of mastitis. The udders of twenty-four cows which had passed through one or more lactations all contained mastitis streptococci, but in six of these no organisms could be isolated from the fore-milk previous to slaughter. Of twenty-one virgin heifers and calves, eleven were found to harbour these organisms in their udder tissue. The findings of this investigation lose some of

their significance when the bacteriological protocols are analysed. For instance in the udders of the heifer group, only three of the eleven animals yielded cultures with the commonly accepted characters of mastitis streptococci. The remaining strains differed in that they usually fermented raffinose and inulin, features which are usually regarded as characteristic of *Str. bovis*. Similarly with many of the strains isolated from the udders of cows the same failure to recognize the usual reactions of these organisms is evident.

Hastings & Beach (29) have shown that it is difficult to maintain a herd free from individual animals producing milk of abnormal composition even though *Str. agalactiae* infection is absent. Thirty-one heifers which had had no contact with other stock were observed during their first lactation, and during this time one or more samples from twenty-three animals satisfied the criteria of abnormality, namely, a *p*H value above 6·8, a chlorine value above 0·15 % and a catalase value in excess of 50 %.

By vaccination and by chemotherapy. A study of the possible prophylactic or therapeutic value of formalized vaccine on the course of streptococcus mastitis was made by Hucker & Hansen (30) in four herds containing 102 animals. Approximately one-half of these were treated with periodic intramuscular injections of vaccine prepared from stock and from freshly isolated streptococcus strains while the other half remained untreated. Prior to vaccination, weekly quarter samples were analysed by the following tests—bacteriological test, leucocyte count, reaction to brom-thymol-blue and physical appearance; after treatment the same tests were carried out at intervals for a period of 2 years. It was found that vaccination did not produce significant protection as judged by the reaction of the milk nor was any immunity stimulated when comparison was made in the leucocyte counts of the same animals before and after vaccination and with those in the untreated group. By the same criteria, vaccination was found to have little or no therapeutic action in the treatment of latent udder infections.

Steck *et al* (31) estimated the value of vaccination following the administration of a living autogenous vaccine to ten cows in an infected herd by determining the streptococcus count of their milk samples before and after vaccination. Examination of the milk at regular intervals showed no difference in the numbers of organisms excreted and the authors conclude that vaccination did not affect the course of the disease in any way.

Steck (32) records the success obtained in the control of streptococcus mastitis in eight herds by infusion of infected udders with acridine compounds and by elimination of infected animals. Various therapeutic substances were tried but trypaflavine was preferred. Helm (33) tested the therapeutic value of "entozon" infusions in thirty-three cows affected with chronic mastitis. In only two of these was the treatment successful although two to three injections were administered.

Recently the value of udder infusion with solutions of entozon (1 : 1250) and of acriflavine (1 : 10,000) in the treatment of streptococcus mastitis has been investigated by Stableforth & Scorgie (34). The treatment was administered on two occasions by injecting the solution into the infected quarter, where it was allowed to remain for 5 min. in lactating cows and for 24 hr. in dry cows. Success, as determined by bacteriological tests, was obtained with entozon in forty-three out of sixty-three (71·7 %) quarters affected with latent mastitis and in thirteen out of thirty-seven (35·1 %) quarters clinically affected, while the corresponding figures with acriflavine were twenty-six out of twenty-nine (90 %) and nine out of twelve.

The various sulphanilamide compounds which have recently been found to yield such success in the treatment of human streptococcal infections have not yet been thoroughly tested on the corresponding diseases of animals, particularly bovine mastitis. Two preliminary trials have, however, been recorded. Allot (35) tested the

therapeutic value of sulphanilamide on three cows affected with chronic mastitis. Doses of 30 g. were administered orally twice daily and the treatment continued over periods varying from 5 to 12 days. It is claimed that there was a definite response to treatment as judged by clinical and laboratory tests. The results of Johnson & Miller (36) with this compound are not favourable. Two cows received six 75 gr. doses and four cows ½ oz. doses twice daily for 8 days. The results showed that the drug had no therapeutic action and it was ineffective in preventing the spread of infection to other quarters of the same animal.

Mastitis due to staphylococci

The literature dealing with the role of staphylococci in the production of mastitis has been reviewed by Minett (37). According to this author the disease due to these organisms may be of an acute inflammatory nature or it may assume a more chronic form with symptoms similar to those produced by *Str. agalactiae*. Five cows affected in this way with haemolytic staphylococci were studied and a complete account is given of the clinical findings and the pathological changes in the affected quarters of three cows. The disease was characterized by a diffuse induration of the gland with some decrease in the milk yield and qualitative changes in the milk such as excessive alkalinity, increase in leucocytes, and a lowered specific gravity and solids-not-fat content. Quarter samples of 415 cows belonging to five herds were examined and *Staph. aureus* was found in 145 (34·9 %); in one herd there was clinical evidence of infection in nineteen out of twenty quarters found to be harbouring the organism. For its cultivation the importance of blood agar containing sheep or ox blood is stressed because it produces characteristic haemolytic colonies in this medium. The relationship between staphylococcus infection of the udder and the presence of antitoxin in the blood of cattle has also been investigated by Minett.

Little & Foley (38) have shown that certain staphylococci isolated from the milk of normal udders, from fatal cases of mastitis and from superficial abscesses of the skin of the udder comprise a definite group possessing common characteristics. All are composed of small spherical cocci, producing moderate zones of haemolysis on blood agar; they are incapable of utilizing ammonium phosphate as the sole source of nitrogen and are pathogenic for mice. Following the injection of fairly large numbers into the udder, a very acute mastitis ensues and constitutional symptoms are produced. It appears that fresh milk has an inhibiting action on a pure culture of the organism and the authors suggest that mastitis develops only when the concentration of inhibiting substances within the udder is low.

Influence of mastitis on milk yield

The influence of *Br. abortus* infection and of mastitis on milk yield has been studied by Minett & Martin (39) in cows belonging to two dairy herds. A total of 373 lactations was studied, 180 in Ayrshires and 193 in Friesians. Milk yields, after correction by a standardized method, were compared in groups according to the presence of mastitis accompanied by or free from *Br. abortus* infection (clinical abortion did not occur). The criterion of mastitis infection was mainly based on laboratory diagnosis of the presence of *Str. agalactiae*. The average reduction in milk yield per lactation was 954 lb. due to mastitis and 1685 lb. due to mastitis and abortion infection combined. The percentage fall in yield between the healthy and mastitis-infected Ayrshires was on an average 10·8 and in the Friesians 16·5 in one herd and 19·5 in the other. White *et al.* (40) compared the milk yield of cows while free from chronic mastitis with their performance after infection. The cows of two herds were examined

by direct and indirect tests on the milk. After corrections for age, length of lactation and number of milkings it was found that with cows reacting in one or more quarters to all these tests the average loss of milk was 679 lb. or 6·5 % per lactation in one herd and 559 lb. or 5·7 % in the other. There was considerable variation in the response of individual cows to infection with *Str. agalactiae*; some did not show any reduction in yield while others appeared to recover after having shown an initial loss. A few cows exhibited immediate and drastic reductions in yield which continued until permanently abnormal production was established.

A further investigation on this subject has been carried out by Hucker *et al.* (41) on thirty-five cows examined over a period of 4 years. It was found that infection of the udder did not affect milk production when the only evidence was the presence of mastitis streptococci or of more than 500,000 leucocytes per ml. in the fore-milk. No appreciable effect upon production could be found until infection was sufficiently advanced for the milk to be abnormal in physical appearance. Latent mastitis confined to one quarter did not significantly influence production but there was a reduction when three or more quarters were involved.

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II. CONTAGIOUS ABORTION

Pathogenesis in cattle

While *Br. abortus* in infected cattle is usually found in the uterus, udder and lymphatic glands, various workers have reported the isolation of the organism from other sites. For example, Lübke (42) recovered brucella organisms from the blood of 90 % of infected animals in one herd, but in only 6 % in another herd. There appeared, further, to be no consistent relationship between the blood agglutination titre and the presence of organisms. Bosworth (43) recovered the organism from the urine and faeces of one infected cow and from the urine of another. In the first cow there was very little chance of the urine being contaminated by uterine discharge because a considerable period had elapsed since the last parturition, whereas in the other cow there was a possibility of contamination with uterine discharge resulting from a recent abortion. It is interesting to observe from the work of Fitch *et al.* (44) that *Br. abortus* is not ordinarily present in the vaginal mucus of pregnant infected cattle prior to calving or abortion. Out of a total of 440 examinations of swabs taken from the vagina of fifty-eight infected animals, only seven yielded cultures of this organism and five of these were recovered from cows 24 to 48 hr. before parturition at a time when the uterine seal was broken.

One of the principal reservoirs for *Br. abortus* in the cow is the udder, where according to Sholl (45) it may be responsible for an interstitial mastitis. The organism is excreted in the milk and specific antibodies can be demonstrated.

Stockmayer (46) studied the frequency and duration of *Br. abortus* infection in the milk of recently infected cows. Of thirty-nine heifers artificially infected with *Br. abortus*, twenty-two showed the presence of this organism in the milk at normal calving or abortion. It was found in greatest numbers (up to 200,000 per ml.) in the milk at the beginning of lactation, declining as lactation advanced until the excretion became intermittent. During the dry period there was an increase in numbers and in most cases the organism persisted until the second lactation. The agglutinins in the whey of infected udders were found to reach a concentration of 1/10,000. It is generally accepted that when *Br. abortus* is excreted in the milk of cows, the agglutinins in the blood must be present in a fairly high concentration. Occasionally, however, it has been reported that the organism may be isolated from the milk of cows which

do not react to the agglutination test. Henry *et al.*(47) correlated the blood and milk agglutination titre with the excretion of organisms in the milk of twenty cows. It was found that the organism was never present in milk having a titre of 1/50 or less, while nearly all those animals with blood titres of 1/200 or more harboured the organism in the milk. Doyle & Becket(48) examined milk samples for the presence of *Br. abortus* from non-reacting cows belonging to seventeen infected herds. The organism could only be recovered from the milk of two out of 309 non-reacting cows, i.e. cows which did not show a blood titre of 1/25 or over.

Barrat(49) has reported an examination of samples of bulk milk for the presence of *Br. abortus*. Of 157 samples of ordinary raw milk the organism was recovered from thirty-five (21 %). Only one sample of certified milk was examined and this was infected, while the organism was absent from five samples of pasteurized milk.

In the female bovine, *Br. abortus* tends to set up a permanent infection. Mitchell & Humphreys(50) investigated the duration of infection by serological tests on 227 cattle contained in three heavily infected herds. The tests were repeated at regular intervals over a period of 1–4 years. It was found that while infection in calves was transient, in mature cattle only 3 % returned from a positive to a consistently negative reaction.

Br. abortus infection in the bull may cause an orchitis and vesiculitis and in this state the semen may be infected. Infection may be directly transmitted to cows in this way during service or the bull may act as an intermediate carrier of infection from infected cows to clean cows. A pathological condition in the bull due to this infection is not frequently encountered but the spread of the disease during service may be of importance. Thomsen(51), investigating this question, found that the spread of brucella infection in cattle could be caused by the bull under experimental conditions. Fifteen heifers were served by bulls whose preputium had been injected with a suspension of infective material. Five heifers subsequently aborted and five calved normally, while five were not fertilized. The author states that these experiments hardly permit decisive conclusions regarding transmission of brucella infection by service under natural conditions since the infecting dose was considerably in excess of that which would occur normally. From similar experiments Moretti(52) concluded that bulls are unlikely to spread infection unless definite lesions exist in the reproductive organs and in this connexion Delez(53) was successful in producing pathological lesions in the genitalia of bulls by inoculating *Br. abortus* culture.

Pathogenicity of Br. abortus *for other animals*

While the bovine animal is the natural host of *Br. abortus* there is some evidence that other animals are either naturally infected with this organism or are susceptible to experimental infection.

In the horse, the organism may be found in chronic abscess conditions such as fistulous withers or poll evil. Further evidence relating to the isolation of *Br. abortus* from a number of clinical cases has been given by Schoop(54), Ebner(55) and Duff(56). Duff found that in most of the cases examined by him there was a history of contact with cattle infected with contagious abortion.

McNutt(57) tested the sera of 1547 pigs for the presence of *Br. abortus* agglutinins and found that 2·3 % reacted; in a few reacting sows the infection was associated with a metritis. Köbe(58) found that brucella infection was responsible for mortality in young pigs.

Natural infection of the dog has also been recorded and the diagnosis has been based on the presence of specific agglutinins in the blood. Pathological lesions due to this organism, however, have rarely been reported. Feldman *et al.*(59) examined

the sera of 500 adult dogs and found the presence of specific agglutinins in fifty-two (10·4 %), but the presence of brucella organisms could not be detected in any of these reacting animals. Whitby *et al.* (60) recovered a strain of the bovine type of *Br. abortus* from the epididymis of an old dog where it was responsible for a suppurative condition.

Hitherto, the laboratory animal most commonly used for experimental purposes and for the isolation of *Br. abortus* from naturally infected material has been the guinea-pig but in recent publications it has been suggested that mice could be used, especially where large numbers of experimental animals are required. Feldman & Olson (61) found that the latter were susceptible to infection and for this reason may be considered suitable test animals—a finding which is supported by the experiments of Singer-Brooks (62). Scorgie (63), however, found that mice were much less susceptible to infection than guinea-pigs and states that they cannot replace the latter for diagnostic and experimental procedures.

Viability of Br. abortus. In order to determine how long *Br. abortus* can remain viable in aborted foetuses and membranes Bosworth (64) exposed infected material under natural conditions during summer and winter periods. The experiments showed this organism to be very resistant to putrefaction and desiccation and for this reason pastures cannot be considered safe for grazing after an abortion has occurred until they have been left vacant for a period of at least 6 months.

Diagnosis

The agglutination test. In order that the interpretation of the agglutination test may be the same in the hands of different workers and that the results may be constant from time to time, it is essential that the technique and reagents employed should be strictly standardized. Stableforth (65) has compared the methods and diagnostic criteria of the agglutination test as adopted in six different countries with the standard adopted by the Agricultural Research Council in Great Britain. The technical methods of conducting the test were found to vary in most of these countries and these differences were related to a number of factors, viz. the degree of opacity of the bacterial suspension used, the agglutinability of the organism, the method of standardizing the antigen and the technique of carrying out the test. Finally, there was no uniformity in the interpretation of the results obtained. Fifty sera were submitted to the agglutination test with suspension obtained from each of the six countries and the results obtained showed a striking divergence in the final diagnosis as it is customarily interpreted in these countries. For example, according to the British standard, twenty sera were classified as positive, twenty-three as doubtful and seven as negative; the corresponding figures for Germany were four, two and forty-four, while for the U.S.A. they were twenty-six, twenty-four and none. Stableforth (66) has described the preparation of a suspension of *Br. abortus* which is standardized to a uniform agglutinability by means of a dried anti-abortus serum. Such a serum is prepared by drying the serum of a cow naturally infected with *Br. abortus* to constant weight over phosphorus-pentoxide under reduced pressure. The antibody content of this serum is slightly lowered by drying but subsequently remains constant for a considerable period. Standard suspensions of *Br. abortus* are prepared by adjusting the opacity so that a constant end-titre is obtained when tested with dilutions of the dried serum.

In the United States considerable attention has been given to the significance of doubtful reactors to the agglutination test, i.e. agglutinins in serum titres of 1/100 or below. (In Great Britain the reaction is interpreted as doubtful in titres of 1/20 or below.) Fitch *et al.* (67) examined the blood sera of eighty-six such reacting animals

which were maintained under supervision for at least one calving period. During this time frequent tests were made on the agglutination titre of the blood, and for the presence of *Br. abortus* in the milk, placentas and vaginal discharges. During this time the animals were housed and pastured with animals known to be free from infection. Only three animals became positive to the agglutination test during the experiment and two of these aborted. The authors state that a definite rule for the disposition of all animals having suspicious titres for Bang's disease is impossible on the result of a single test and that it is necessary to have the results of a series of tests over a considerable period together with information regarding the duration of pregnancy and an adequate herd history before proper deductions can be made. Similar observations have been made by Donham & Fitch (68) and by Ranney (69).

Other serological tests. In addition to the agglutination test, the presence of *Br. abortus* antibodies in the blood can be demonstrated by the complement-fixation test. Other tests which have been employed are the Sachweh flocculation test and the Meinicke flocculation tests. The value of these tests for diagnosis has been investigated by Pröscholdt (70), by Feldt (71) and by Seeleman & Green (72), but it is difficult to assess the value of the comparisons made with the agglutination test which was regarded as positive only when a definite reaction at 1/100 was present.

Welch & Marsh (73) report that a whole-blood agglutination test is practicable for the diagnosis of contagious abortion and is particularly suitable where a rapid result is required, as for instance in the detection of reactors in range herds. The technique of performing this test is described and it appears that the results obtained compare favourably with those of a standard serum test.

The biological test. The difficulty of isolating *Br. abortus* from "dirty" milk by means of guinea-pig inoculation has been the subject of experimental work by Pullinger (74). According to this worker the explanation appears to lie (in certain cases) in the presence of tubercle bacilli in the inoculum. Following the simultaneous inoculation of virulent tubercle bacilli and *Br. abortus* into guinea-pigs, infection due to this latter organism failed to become established, whereas control animals inoculated under the same conditions with brucella culture alone became infected. It is suggested that the mononuclear cell reaction stimulated by tubercle bacilli destroys *Br. abortus*.

Immunization

Considerable attention is now being given to the possibility of immunizing cattle by means of non-virulent strains of *Br. abortus*. Vaccination with such strains would possess an obvious advantage over the use of virulent strains if it could be shown that they did not localize within the udders of lactating cows. According to observations made by Norris (75) it is clear that pathogenicity tests are required to establish the virulence of strains which have been subcultured for as long as 15 years. Five such strains possessed sufficient virulence to produce infection which resulted in abortion after injection into pregnant heifers. This worker states that it is undesirable to vaccinate pregnant animals with living cultures of so-called avirulent or old strains of *Br. abortus* as it is likely to result in a small percentage of the animals aborting or showing udder infection or even becoming permanent carriers. Before attempting to immunize cattle with any living *Br. abortus* culture it is desirable that a thorough preliminary study should be carried out in susceptible animals such as guinea-pigs to determine the virulence of the strain. A series of experiments of this nature has been recorded by McEwen & Roberts (76). A high degree of resistance to subsequent infection with virulent culture was demonstrated in male guinea-pigs vaccinated with a non-virulent strain. In one experiment 90 % of the vaccinated group resisted infection, whilst a control group all became infected. The uterus of

female guinea-pigs was found to be highly susceptible to infection but when vaccinated before pregnancy with a non-virulent strain a high degree of resistance to subsequent infection was attained. This immunity in the guinea-pig did not depend on the persistence of the vaccine strain within the body. The infectivity of this non-virulent strain was subsequently tested by McEwen (77) in non-infected pregnant cattle. The subcutaneous inoculation of thirteen first-calf heifers and two cows did not produce uterine infection or abortion nor did the vaccine strain localize in the udder. The immunizing value was tested in eight herds in which contagious abortion was active at the beginning of the experiment, and a complete record was kept for 3 years of the breeding history of the herds and of the agglutination titres of each animal vaccinated. The results showed that of 203 vaccinated animals, 5·9 % became infected with *Br. abortus* during the first year after vaccination; of 160 of these animals observed during the second year 1·6 % became infected and of the animals observed during the third year none became infected.

A similar investigation has been reported by Haring (78). Ninety-three heifers in a herd affected with contagious abortion were vaccinated before service with a *Br. abortus* strain of low virulence. For a period of 4 years the results were beneficial in retarding the spread of the disease and there was no tendency for the vaccine strain to spread to unvaccinated cattle. At 108 parturitions of animals in the unvaccinated group, *Br. abortus* was found in the milk of twenty, while at ninety parturitions of those in the vaccinated group only two excreted the organism in the milk and it is believed that these were infected before vaccination. Meyer & Huddleson (79) used a vaccine prepared from a living non-virulent culture which did not produce agglutinins in animals which had received large and repeated doses and this vaccine appeared to confer a significant resistance to infection.

It has generally been found that immunity is not conferred by vaccines which have been killed by heat. Priestley (80) has shown that the difference in immunizing value of living and dead cultures is not primarily related to the loss of a heat-labile antigen in the latter.

Experiments have been conducted with a view to determining whether it is possible to immunize an animal to *Br. abortus* infection by means of a vaccine in a fatty base, as is sometimes practised in cases of tuberculosis, Johne's disease and diphtheria. Velu & Zottner (81, 82) showed that an oily excipient prevented the dissemination of the organism but did not interfere with the production of antibodies in rabbits, while in bovines a mild systematic reaction ensued accompanied by a strong serologic reaction but rarely by abortion. Dubois (83), who made similar observations, also found that a vaccine made from heat-killed culture suspended in a fatty base was of much inferior antigenic value to a living vaccine.

Further studies on the immunization of cattle at an early age with a view to protecting them as adults have been recorded. Delez (84) gives an account of eight heifers which had been vaccinated as calves with a strain of *Br. abortus* of moderate pathogenicity. They were injected with a virulent strain at the fifth month of gestation and three unvaccinated controls were similarly treated. While the vaccinated group all had full-time pregnancies, two controls aborted and one calved at full time. The persistency of agglutinins in the blood following vaccination of young cattle with a low virulent strain of *Br. abortus* has been studied by Butler *et al.* (85). In a group of heifers vaccinated at 6–12 months of age the agglutinins produced by vaccination were reduced in 12 months to such an extent that no reaction occurred at a 1/200 dilution—at the end of 2 years 77 % of the heifers were negative at 1/25 while none reacted at a dilution higher than 1/50. These results indicate that *Br. abortus* does not become established in the body following injection in early life. This finding has been verified by Tompkins (86).

Control

By segregation methods. A very interesting paper dealing with the control of contagious abortion in a herd and with the effect of *Br. abortus* infection on its breeding history has been presented by Miller *et al.* (87). In a dairy herd containing sixty-five females negative to the agglutination test and eighty-two reactors, removal of the latter group to distant premises did not prevent the spread of the disease. For the latter purpose it was necessary to exercise very strict supervision of all communication between the two groups of animals and to remove all cows at parturition to separate boxes until they had shown a negative blood test. A study was made of the fertility of the animals in the clean section of the herd, of those in the original infected section and of those periodically removed from the clean section on account of infection. It was found that in eighty cows belonging to the last group, more services for a conception were required than for the other groups and that the incidence of abortion was higher. The authors attribute the lowered fertility of this group to the effect of infection recently acquired.

By eradication. In the U.S.A. the eradication of contagious abortion is undertaken on a voluntary basis and most of the work is conducted in individual herds. In several States, however, arrangements have been made to conduct a programme in a systematic manner in a given area such as a county, and such co-operative efforts to reduce infection within these areas have been very successful. Wight (88) has presented figures up to October 1937 regarding the number of herds under control: about 802,000 herds containing over eight million cattle were tested for Bang's disease and the average percentage infection was found to be 5·0. In several States undertaking control in areas not more than 2 or 3 % of the animals were infected.

The progress made in the eradication of contagious abortion in Canada has been described by Hilton (89). In this country assistance is offered to stock owners in controlling this disease by conducting blood tests of their cattle in order that infected animals may be segregated or slaughtered as circumstances warrant. The number of owners taking advantage of this assistance is steadily increasing and, in 1937, 1650 herds comprising 42,900 cattle were under supervision.

A voluntary eradication scheme was initiated in Norway in 1935. Of 2741 herds originally infected 75 % were regarded as free from disease by the end of 1936 and further progress was made during 1937. In Switzerland a similar scheme is also available and provision is made for free blood testing, State assistance being given in the necessary replacements of infected animals and official recognition of abortion-free herds being accorded.

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III. TUBERCULOSIS

Pathogenicity of *Mycobacterium tuberculosis*

The pathological and bacteriological findings in naturally acquired tuberculosis in ten different species of animals has been described by Griffith (90). The tubercle bacilli obtained in culture from five species (bat, bear, bison, hedgehog and mink), all cases of fatal tuberculosis, were of the bovine type. The bacilli from a case of localized glandular tuberculosis in a goat and one of tuberculosis in the pancreas in a kangaroo were of the avian type. The bacilli from a case of generalized retrogressive disease in a horse, of minimal thoracic disease in a calf and cutaneous glandular tuberculosis in a parrot were of the human type. The writer states that these results amplify the evidence already published which shows that each of the three types of tubercle bacilli (bovine, human and avian) is able to cause natural tuberculosis in

many different species of animals other than that which is its normal habitat. Wells(91) has reported a disease of the field vole in which the pathological lesions resemble those of tuberculosis and from which organisms similar to *Mycobact. tuberculosis* in growth and morphology have been recovered.

A series of experiments to determine the viability of an avian strain of tubercle bacillus alone in soil and in association with soil micro-organisms was conducted by Rhines(92). Pure culture was added to flasks of sterile soil and to soil containing various soil bacteria. After storage at room temperature for varying periods the number of tubercle bacilli in each was determined by plating on a special medium. It was found that the bacillus multiplied during storage for 97 days (the maximum period of observation) in association with the other cultures. In toluol-sterilized soil the avian tubercle bacillus survived for 3 months, but in unsterilized soil the number of viable organisms was reduced in one month.

The fact that the tubercle bacillus possesses considerable resistance to conditions of exposure in soil would suggest that infection of animals by contaminated pastures might be an important factor in the dissemination of tuberculosis. The experiments of Maddock(93) on this subject do not, however, support this hypothesis. An area of pasture was infected in a natural manner by grazing calves which were receiving massive doses of bovine tubercle bacilli in their food. After 3 weeks' grazing, these calves were removed, the dung spread evenly and the area divided into three small plots. Healthy calves in pairs were then allowed to graze in each of the plots for 3 weeks and then removed to a clean paddock. After three separate negative tuberculin tests had been made the calves were slaughtered 7, 6 and 5 months later. Careful post-mortem examination failed to reveal the presence of tuberculous lesions. A second experiment was carried out on the same lines except that the source of infection was from the dung of two naturally infected cows. This time three calves were grazed on each of two plots for periods of 53 and 24 days respectively. When these calves were slaughtered after being kept for 1 year on clean pasture, no tuberculous lesions were found. While these experiments lend additional support to the belief that infection in tuberculosis is principally contracted by direct contact, it should be pointed out that the periods of grazing were of short duration and that these experiments were carried out only in the summer.

In the recent literature many reports on the pathogenesis of tuberculosis in calves have appeared. Inhalation is usually regarded as the usual method of natural infection because evidence of the disease is most frequently found in the pleural cavity. Furthermore the administration of tubercle bacilli by the oral route has been stated to give rise to tuberculosis of the intestinal tract (Nieberle(94), Dobberstein & Wilmes(95)). In the experiments of Wilkie *et al.*(96, 110) however, where naturally infected milk was fed to calves the intestinal tract was not involved and the lesions present were confined to the pleural cavity. These experiments show the importance of ingestion as a primary mode of infection.

Diagnosis

By cultural and biological methods. Various media are in use for the primary cultivation of the tubercle bacillus. Witte(97) found that Petragnani's medium was suitable for this purpose especially after enrichment with broth and potato or pea flour. It was found desirable to seal culture vessels in order to obtain the optimal gas tension for growth. Maximum growth was obtained under conditions of symbiosis with *Bacillus Simplex*, the growth being visible after 10–20 days' incubation. Ruddock-West & Alpe(98) recommend the following technique for cultivating the tubercle bacillus from milk. The sediment obtained by centrifuging at 3000 r.p.m. for 20 min. is

treated with 20 % H_2SO_4 for 3 min., neutralized with NaOH and sown on a modified Löwenstein-Jensen medium. Cultures are incubated for 10–14 days when they are examined for the presence of growth. Out of 904 samples which were negative on microscopic examination forty-five were positive by the cultural method. Likil(99) states that cultural examination on Petragnani's medium affords a rapid method of diagnosis but that it is preferable to use this test in conjunction with the biological test.

The value of the biological test using either one or two guinea-pigs for inoculation has been investigated by Mattick & White(100). When 3346 samples of bulk milk were examined by injecting one guinea-pig, eighty-four reinvestigations were necessary for each 1000 samples as a result of deaths from intercurrent infections, and sixty-two infected samples were undetected. When two guinea-pigs per sample were used, only seven reinvestigations following the death of both animals were necessary and only four infected samples were not diagnosed.

Maitland(101) has described a technique for the rapid detection of tubercle bacilli in milk which depends on the recognition of "cell groups" in centrifuged sediment. Examination by this method appears to compare favourably with results obtained by the biological test.

By clinical methods. In Germany and Sweden where the eradication of tuberculosis in cattle by the removal of clinically affected animals is practised, attention is paid to the detection of so called "open" tuberculosis i.e. tuberculous conditions where the animal is actually excreting tubercle bacilli. Such conditions may exist when there is disease of the lung, uterus or udder. The various methods used in these countries for examining sputum, uterine discharge and milk have been described by Brook(102). This author states that it has been found that these methods fail to detect 30 % of the infected animals, and this failure is said to be due to the fact that tubercle bacilli are excreted intermittently. Wagener(103) states that the elimination of animals found to be infected by examining the bronchial mucus is not effective in reducing the incidence of tuberculosis.

By the tuberculin test. The disadvantages associated with the use of concentrated broth tuberculin in the intradermal test have been described by Glover(104). These disadvantages are chiefly due to the fact that it is difficult to remove non-specific substances which are responsible for skin reactions in non-tuberculous animals. These non-specific reactions are minimized by the use of tuberculin prepared from the products of growth of tubercle bacilli in a synthetic medium, Glover(105). Tuberculin prepared from such a medium contains, in addition to the active principle, the unused mineral components of the medium together with other products which may be elaborated by the organism during its growth but are not necessarily associated with tuberculin activity. The following method has been devised, therefore, for preparing a purified tuberculin. Suitable strains of the tubercle bacillus are cultivated in a simple synthetic medium for 8–10 weeks. The culture is then steamed, filtered and the active principle separated by saturating with ammonium sulphate. The precipitate is then collected, redissolved in glycerolated solution so that it is twenty times the concentration of the original fluid, passed through a bacterial candle and preserved with an appropriate amount of merthiolate. The final step consists of a test for potency in tuberculous cattle, comparison being made with equivalent amounts of standard tuberculin. An inexpensive synthetic medium which is suitable for the production of large quantities of tuberculin has been described by Wong & Weinzirl(106). Its production involves approximately one-third of the cost of that of Long & Seibert's asparagine medium. Maximum growth of the tubercle bacillus in this ammonium malate medium with 1 % glucose added may be obtained in 4 weeks, when the same yield of tuberculin can be obtained as in the asparagine medium but in approximately half the time.

The technique and interpretation of the double intradermal test have been well described by Jordan (107). In differentiating reactions it has been found that a positive diffuse swelling invariably grips the underlying tissue. Doubtful reactions are occasionally experienced, and animals presenting such reactions should be kept under observation and retested in a short period. Douglas (108) states that in his experience doubtful reactions have been more frequently encountered since the practice of testing all the animals in a herd was introduced under the Tuberculosis (Attested Herds) Scheme, the reactions in question being found chiefly in young animals. In view of the fact that there is no adequate explanation for the occurrence of such anomalies, Douglas is of the opinion that doubtful reactors should be treated as infected and eliminated from the herd.

A comparison of the diagnostic value by the intradermal method of synthetic tuberculin and standard old tuberculin has been made by Tweed (109). Twenty-six out of thirty-six animals gave definite reactions to both tuberculins, and post-mortem examination confirmed the presence of tuberculosis in twenty-five of these animals; no biological test was carried out on the other reacting animal. The remaining animals were negative to the tests except two which gave a doubtful reaction to old tuberculin but were negative to synthetic medium tuberculin; at post-mortem no visible lesions were found in these. It was found that the character of the swellings induced by the synthetic product facilitated the interpretation of the reactions when compared with old tuberculin. Verification of the double intradermal test using synthetic medium tuberculin by post-mortem examination has also been carried out by Edwards (110) who applied the test to seventy-three calves. Twenty-five of these reacted to tuberculin and the presence of tuberculosis was confirmed in twenty-three; in the remaining two reactors naked eye search failed to reveal tuberculous lesions in one, while in the other the inoculation of glandular material into guinea-pigs did not show the presence of tubercle bacilli. In those animals free from disease the injection of tuberculin did not give rise to more than a small pea-like induration, whereas in the majority of affected calves an intense reaction characterized by a diffuse oedematous swelling of the skin was evident 48 hr. after injection.

Non-specific reactions to the tuberculin test

Anomalous reactions to the tuberculin test in bovines may sometimes be due to infection with avian tubercle bacilli or to the presence of a nodular condition of the skin to which the term "skin tuberculosis" has been applied. This latter condition has been principally encountered in America where affected animals have been referred to either as "no visible lesion reactors" or "skin lesion reactors".

Avian tuberculosis in cattle. From time to time various workers have reported the isolation of avian tubercle bacilli from cattle which have shown sensitivity to mammalian and avian tuberculin. In some instances it has been possible to relate the disease to contact with tuberculous fowls. The lesions found in cattle consist of discrete localized foci situated in the lymphatic glands. Glover & Griffith (111) describe the history, post-mortem and bacteriological findings of four animals affected with avian tuberculosis, which belonged to tuberculosis-free herds. Of the four animals only two calves had been reared in contact with infected poultry. These calves reacted to bovine tuberculin but a more pronounced sensitivity to avian tuberculin was shown. At post-mortem the lesions in these calves consisted of tuberculous foci confined to the mesenteric glands and subsequent verification of the acid-fast bacilli recovered proved that they belonged to the avian type. McCarter *et al.* (112) state that in Wisconsin, which has been an accredited state for bovine tuberculosis since 1932, 0·24 % of cattle tested in 1935 reacted to tuberculin. Sensitization to the

avian tubercle bacillus was responsible for a part of this residuum of reacting cattle. Of the tuberculous lymph nodes of twenty-eight reacting cattle examined by cultural and biological methods three were found to be affected with avian bacilli. In a self-contained herd examined by Feldman & Schlotthauer (113) which had been subjected to tuberculin tests for 11 years, twenty-one animals reacted but in no instance was the bovine type of tubercle bacillus obtained although four strains of the avian type were isolated from three animals. A flock of fowls in which tuberculosis was known to exist was maintained in direct contact with the cattle.

Skin tuberculosis. The present state of knowledge regarding skin tuberculosis in cattle has been reviewed by Brook (114) and by Robertson & Hole (115). The condition constitutes a serious problem in the United States and Canada. Many herds in the former country have failed to become accredited owing to its presence or have lost their status due to animals reacting to the tuberculin test. In Canada recently the effect of the disease on tuberculin reactions has necessitated a special survey of the problem. The main features of the condition may be summarized as follows. The pathological lesions are confined to the skin where a varying number of nodules are found in and under the skin, mainly of the legs though occasionally the shoulder and flank may be involved; there are no clinical signs of inflammation. Frequently these nodules contain acid-fast organisms and have a histological structure closely resembling that of a tuberculous focus. It has been found, however, that guinea-pigs inoculated with this tissue do not succumb to tuberculosis. Furthermore, attempts to cultivate the organism on media which favour the tubercle bacillus have usually been unsuccessful. Successful cultivation of acid-fast organisms from material from two cases of skin tuberculosis has been recorded by Crawford (116). The organisms were isolated by culture from the normal spleen of guinea-pigs which had been infected with material from affected tissue. Subsequently they proved to be non-pathogenic for guinea-pigs, rabbits and fowls, but when injected subcutaneously into cattle they produced lesions simulating typical skin lesions. Pendergast (117) failed to demonstrate the presence of concomitant tuberculosis in the tissues of animals showing lesions of the skin.

The recent increased attention to the eradication of tuberculosis in Great Britain has resulted in the recognition of animals affected with skin tuberculosis in herds which have been considered free from tuberculosis. Robertson & Hole (115) have recently presented a brief description of twelve cattle affected with the condition in this country. They had shown inconsistent or inconclusive reactions to the tuberculin test and the only factor accountable for sensitization to tuberculin appeared to be the presence of skin lesions. Acid-fast organisms were present in the lesions of eleven cattle and the histological structure was similar to that of a tuberculous granuloma. It was not found possible to recover the organism by cultural or biological methods. These workers admit the possibility of the lesions being due to an atypical form of *Mycobact. tuberculosis* which may not necessarily be the primary cause of the disease but may be a secondary invader of a wound or abscess; this organism may, however, sensitize cattle to tuberculin.

Kelland *et al.* (118) describe the eradication of tuberculosis from a herd where a considerable number of anomalous reactions to the tuberculin test occurred. These could be attributed either to infection with skin tuberculosis or avian tuberculosis. Altogether twenty-six animals affected with the former condition were encountered and in these the tuberculin reactions were usually smaller and rather less oedematous than specific reactions obtained with bovine tuberculosis. Fourteen heifers which showed a nodular condition of the skin of the legs failed to react after isolation for 60 days.

It would appear from the literature quoted that the problem of skin tuberculosis

in cattle deserves to be closely studied in the future and more particularly the factors concerned in the development of the allergic state.

Immunization

A brief survey of the various methods which have been advocated for the induction of immunity to tuberculosis has recently been presented by Glover (119). Referring to vaccination with B.C.G. (Bacille-Calmette Guerin) he states that the innocuity of the vaccine has been verified by experimental and field observations carried out in several European countries. For instance, Oatway & Steenken (120) state that B.C.G. is considered to have become increasingly difficult to dissociate by recognized methods into virulent (S) variants after further years of subculture. Rosenthal (121) found that the inoculation of guinea-pigs and rabbits resulted in the formation of tubercles and sensitivity to tuberculin but there was no evidence of a progressive tuberculosis. Further, passage of B.C.G. through a series of animals did not enhance its virulence.

Several reports of experiments dealing with the immunization of laboratory animals have been recorded. Clawson (122) has shown that a definite resistance to virulent infection was developed by vaccinating rabbits and guinea-pigs with B.C.G. and that the concentration of antibodies in the blood was uniformly increased in vaccinated animals. Daranzi (123) found that a slightly more resistant state was produced in guinea-pigs. Schwabacher & Wilson (124) in a carefully controlled series of experiments in guinea-pigs found that under suitable conditions vaccination with B.C.G. may confer a certain amount of protection, in occasional animals even complete protection, against subsequent infection with virulent bacilli. The immunity conferred was, however, of a comparatively low order and probably not superior to that produced by suitable injections of dead bacilli. Buxton & Glover (125) have examined the feasibility of inducing an immunity in calves against tuberculosis by the subcutaneous inoculation of B.C.G. or of dead tubercle bacilli in oily excipients (liquid paraffin or olive oil). Three out of four calves vaccinated with B.C.G. proved to be resistant to virulent infection while the fourth showed slight involvement of the mesenteric glands. The results of administering dead vaccine by the same method were not encouraging since only one calf out of four was completely immune. The successful application of B.C.G. for the control of tuberculosis under field conditions has been recorded by Gerlach (126) and by Lours (127).

The resistance conferred by injecting animals with tubercle bacilli killed by heat has received considerable attention and has been compared with that following the administration of B.C.G. Branch & Enders (128) injected guinea-pigs with tubercle bacilli which had been killed by heat and tested the resistance to virulent infection by comparing the survival time with that of unvaccinated control animals. It was found that vaccinated guinea-pigs survived 120–180 days longer than control animals. In a report of the Scientific Committee of the Milan Institute for anti-tuberculous vaccination (129) experiments were undertaken to compare the value of B.C.G. and virulent bovine tubercle bacilli killed by heat as agents for the immunization of calves against tuberculosis. Four animals only were involved and the conclusion drawn was that B.C.G. conferred a higher degree of resistance than the killed bacilli. In a well-controlled series of experiments conducted by Opie (130), it was found that heat-killed tubercle bacilli significantly increased the resistance of rabbits to virulent infection and that this protection was only slightly less than that conferred by B.C.G.

Eradication

Attention will be confined to a review of the position in those countries where the tuberculin test has formed the basis of the eradication policy. A co-operative tuberculous eradication programme has now been in existence for 20 years in the United States, and White (131) presents statistics dealing with the progress of the scheme. During 1937 over 13 million cattle were tested with tuberculin, and the percentage of reactors found was 0·7 (the same figure was obtained in 1936), this being the lowest figure attained since testing began in 1917. The number of accredited herds has remained practically constant during the last three years at a total of 272,000, containing over 3½ million cattle. Four States have been added to the list of those in which all the counties are in a modified accredited area, i.e. where the incidence of tuberculosis is less than 0·5 %. For this purpose, in New York State alone, nearly one million cattle have been slaughtered and the indemnity paid has been about 10 million pounds (Faulder (132)). Only two states, Caroline and South Dakota, have not reduced infection to the accredited figure of 0·5 %, although good progress has been made. White states that only 0·15 % of the total cattle slaughtered in 1937, exclusive of reactors, showed evidence of tuberculosis as compared with 2·3 % in 1916.

In Canada the number of accredited herds in March 1937 was 7827 (Hilton (89)). Owners of cattle who cannot qualify for the accredited herd plan, which demands a certain proportion of pure-bred cattle, may take advantage of the supervised herd plan. Care is taken to eliminate infection and the same methods are followed as in the accredited herd plan but no compensation is paid for reactors although they must be slaughtered under supervision. Under this plan 43,465 herds have been tuberculin-tested.

In Denmark under a voluntary eradication scheme, financial assistance is granted to farmers who are endeavouring to clear their herds by an Act of 1932. According to Plum (133) there are no official figures regarding the number of free herds but Christiansen (134) states that in 1935 eleven islands with about 5915 herds were free from tuberculosis.

Though it has been stated frequently that the incidence of tuberculosis amongst cattle in Great Britain may be as high as 30 % it appears from recent reports that in areas in the more isolated parts of the country this figure may be much lower. Thus Montgomerie (135) found that in four cattle breeding districts in North Wales comprising twenty-eight farms with 594 animals the incidence was 4·4 %. Rabagliati (136) applied the tuberculin test to 171 cattle belonging to seven farms in the West Riding of Yorkshire. Thirteen reactors (7·6 %) were found, ten of which were aged cows; two farms were entirely free from infection. Thomas (137) found that of 690 cattle belonging to twenty-two self-contained herds in Carmarthenshire only eighteen (2·6 %) reacted.

The number of attested herds on 31 March 1938 was as follows: in England 605, the highest number in one county being 57 in Somerset; in Wales 586, of which 324 were situated in Carmarthenshire with 110 and 68 respectively in the adjoining counties of Pembroke and Cardigan. At 30 May the number of such herds in Scotland was 745, of which 363 were located in Ayrshire. The part played by Scotland and Wales, notably by Ayrshire and Carmarthenshire, in supporting the Attested Herd Scheme (which was introduced under the Milk Act of 1934) is highly commendable and demonstrates the success which can be achieved by voluntary effort.

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